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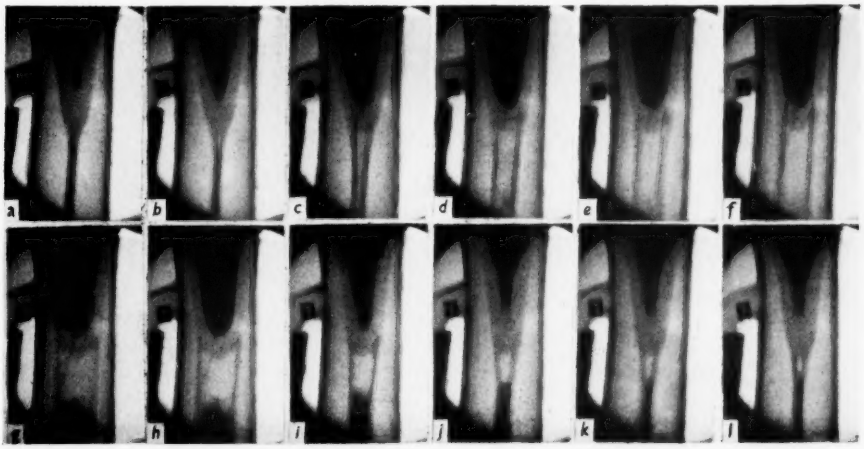
THE JOURNAL OF DAIRY RESEARCH

Vol. 25, No. 2, p. 154

'Cineradiographic observations on machine milking'

By G. M. Ardran, F. H. Kemp, P. A. Clough and F. H. Dodd

The Editors have decided to reproduce figures 1 and 3 of this article on art paper for the benefit of readers. It is suggested these figures be cut out and pasted in their relative positions in the last number.



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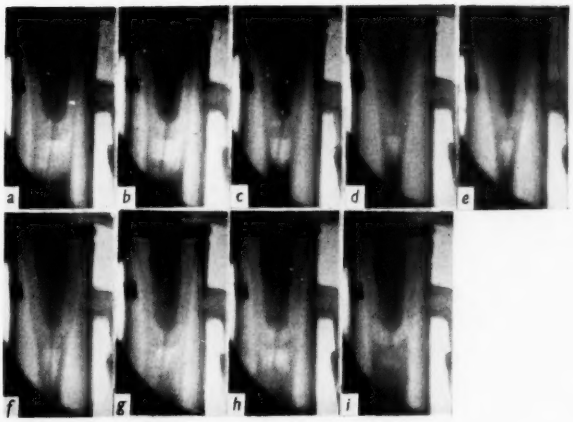

Fig. 3

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636.2.084:612.664.31

723. THE EFFECT OF LEVEL OF CONCENTRATE FEEDING AND TYPE OF ROUGHAGE ON MILK PRODUCTION

BY M. E. CASTLE, D. S. MACLUSKY,* R. WAITE AND J. N. WATSON

The Hannah Dairy Research Institute, Kirkhill, Ayr

Increasing emphasis is being placed on the part which grazed and conserved grass must play in feeding dairy cows (1,2). During the grazing season modern methods of grassland management can ensure an adequate supply of food capable of sustaining high-yielding dairy cows (3,4). When grazing, the cow is able to select an adequate amount of high-quality herbage, but this is not so under winter feeding conditions. Herbage cut for conservation is usually at a more mature stage of growth than herbage used for pasture, and except when artificially dried it suffers substantial losses in feeding value in the conservation process. The stall-fed cow is not able to feed selectively, and if fibrous foods form too large a proportion of the diet there will usually be a reduction in the amount of food consumed as well as in its nutritive value. This is the major difficulty met in attempts to increase the amount of grass products used in winter feeding. The difficulty is minimized when dried grass is fed, and of all grass products dried grass may be expected to effect the greatest saving in the amount of grain or purchased concentrates fed in the winter ration, especially of those high in protein (5). The feeding value of dried grass can be about as high as that of grain, but the cost of milk production from a ration high in dried grass may prove to be greater than from a diet of moderate quality grass feeds when fed with a cheap and largely home-produced concentrate in addition.

To investigate this problem the short-term feeding trial described below was carried out in which a basic allowance of grass silage was supplemented by either hay or dried grass, with a concentrate containing 50% of home-produced foods which was fed at two different amounts for each gallon of milk.

EXPERIMENTAL

Experimental design

Eight Ayrshire cows which had calved from 2 to 11 weeks before the experiment began were used. Four cows were in their second or third lactations and four were considerably older, being in their sixth to ninth lactations. The cows were housed throughout the experiment in a conventional byre and given a period of approximately 2 hr./day for exercise in a bare paddock. After calving, and before the experiment began, the cows were on a diet of silage, dried grass, a little hay and the same concentrate mixture which was fed to them during the experiment.

The eight cows were divided into two groups of four. The cows in group 1 were on average slightly younger than those in group 2, but otherwise the two groups were very similar in stage of lactation, current milk yield and live weight. The four cows in each group were allocated at random to one of four treatments in a balanced complementary 4×4 Latin square (6).

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The experiment lasted 16 weeks, from 28 December 1956 to 19 April 1957, and was divided into four equal periods. The change from one treatment to another was made over a period of 3 days. In calculating the results of the experiment data from the last 3 weeks in each period were used, except for the chemical composition of the milk, which was based on the results obtained in the last 2 weeks of each period.

Feeding

All cows were offered 40 lb. silage per day, and in addition, one of the following four treatments:

A—4 lb. concentrates/gal. and 16 lb. dried grass,
B—2 lb. concentrates/gal. and 16 lb. dried grass,
C—4 lb. concentrates/gal. and 16 lb. hay,
D—2 lb. concentrates/gal. and 16 lb. hay.

The composition of the concentrate mixture and of the grass products is given in Table 1. The approximate starch equivalent and digestible crude protein (D.C.P.) content of the concentrate was calculated from feedingstuff tables (7) and of the roughage from the chemical analyses and Kellner's constants. The silage was fed immediately after the early morning milking, half of the hay or dried grass at noon and the other half after the evening milking.

The weight of concentrates fed to each cow daily at either 2 or 4 lb./gal. of milk was kept constant during each period. The amount fed in period 1 was calculated from the average weight of milk produced by each cow in the week before the experiment began. In periods 2, 3 and 4 the cows were rationed on the equalized feeding principle of Lucas (8), whereby the amount fed in each period was based on the average decline in milk yield of all the cows in the experiment during the preceding periods. Half the weight of concentrates was fed at each milking time.

The silage fed during the experiment had been made from grass cut at the long leafy stage and no preservative had been added to it. The hay had been made on tripods from more mature grass cut at the early flowering stage and was green and moderately leafy. The dried grass was made from leafy ryegrass.

The feeds were individually weighed for each cow every day throughout the experiment. On 4 days in each week the quantities of each feed refused were weighed. In each 21-day experimental period there were, therefore, 12 days on which feed consumption was determined. These are considered to be representative of the period as a whole.

The cows were milked twice a day and their yields recorded at each milking to the nearest $\frac{1}{4}$ lb. Samples of milk from consecutive evening and morning milkings were taken for chemical analysis twice a week from each cow. The evening and morning samples were mixed in proportion to yield and analysed for total solids, fat, crude protein, casein and lactose by methods described previously (9). To detect and if possible control any mastitis which might occur, weekly samples of milk were examined by Dr P. S. Blackburn, M.R.C.V.S., of this Institute, using the differential cell-count method of Blackburn, Laing & Malcolm (10). Foremilk from each quarter was examined bacteriologically each fortnight.

The cows were weighed on one morning each week, at approximately 6.45 a.m., after milking but before the feed of silage.

RESULTS

Feed consumption

Dry matter

The average amounts of hay, dried grass, silage and concentrate dry matter consumed daily on each feeding treatment are given in Table 2. A subtotal of the average daily consumption of grass dry matter is also included.

Table 1. *The composition and calculated feeding value of the concentrate mixture and the roughages*

The concentrate mixture

	lb.	Composition as fed	
Barley (ground)	3	—	
Oats (bruised)	2	—	
Flaked maize	1½	Dry matter (%)	86·3
Dairy cubes (balanced)	1½	Approx. starch equivalent*	68
Tic beans	1	Approx. D.C.P. (%)*	11
Soya bean meal	1	Crude protein (%) (by analysis)	13·6
Locust bean meal	½		
Grass meal (ground)	½		

The roughages

	Hay	Dried grass	Grass silage
Dry matter (%) ...	83·5	85·9	22·1
Percentage of the dry matter			
Crude protein	8·6	16·1	14·5
Ether extract	1·7	4·6	9·6
Nitrogen-free extract	48·4	43·3	37·4
Crude fibre	34·5	23·1	28·3
Ash	6·8	12·9	10·2
Lignin	10·9	6·7	6·9
Total reducing sugars	3·2	11·6	1·7
Approximate starch equivalent†	36	60	55
Approximate D.C.P.†	4·8	11·2	9·5

* Calculated from tables (7).

† Calculated from chemical analyses. Digestibility coefficients from references (15) and (16) and Kellner's constants.

Table 2. *The average consumption of concentrate, roughage, and total dry matter, starch equivalent, digestible crude protein (lb./cow/day) and the dry-matter consumption as a % of live weight*

	Treatment				S.E. of difference between two means
	A	B	C	D	
Hay	—	—	10·5	11·3	±0·31
Dried grass	11·9	12·5	—	—	
Silage	8·2	8·5	8·0	8·2	±0·18
Total grass dry matter	20·1	21·0	18·5	19·5	±0·41
Concentrate	13·5	6·8	13·5	6·9	±0·60
Total dry matter	33·6	27·8	32·0	26·4	±0·61
Daily dry-matter consumption as % of live weight	3·2	2·6	3·0	2·5	±0·06
Approximate starch equivalent (S.E.)	22	18	19	14	±0·50
S.E. as % Woodman standard (7)	141	113	125	100	—
Approximate D.C.P.	3·8	3·1	3·0	2·2	±0·10
D.C.P. as % Woodman standard	124	106	102	82	—

As would be expected, the two different levels of concentrate feeding were responsible for the larger differences between treatments in the daily intake of dry matter. Cows given 4 lb. concentrates per gallon (treatments A and C) ate, on average, 32·8 lb. dry matter per day, but when given 2 lb. concentrates per gallon (B and D) their average intake was reduced to 27·1 lb. When 4 lb. concentrates per gallon were fed the average amount of dry matter from grass products eaten was 19·3 lb., but at 2 lb. concentrates per gallon this amount increased slightly but significantly to 20·3 lb. ($P < 0·01$). The weight of hay eaten was slightly but significantly less than that of dried grass ($P < 0·01$) but the amount of silage eaten was similar on all treatments.

The amount of dry matter consumed per cow per day expressed as a percentage of the average live weight of the cow is also given in Table 2. This value was at a minimum of 2·5% on treatment D and rose to 3·2% on treatment A.

Starch equivalent and digestible crude protein

The average daily intakes of starch equivalent and of digestible crude protein were calculated from the mean consumption of each food and the assumed starch equivalent values given in Table 1, and are presented in Table 2. Because of the empirical nature of the values for the nutrient content of the various rations, the estimates of nutrient consumption are only approximate, but they give an indication of the differences between treatments in the levels of feeding. D.C.P. consumed ranged from 82% of the cows' requirements according to Woodman's standards(7) on treatment D (when hay was fed with 2 lb. concentrates/gal.) to 124% on treatment A (when dried grass was fed with 4 lb. concentrates/gal.).

Animal production

Animal health

Before presenting the results of the yields and composition of the milk it is relevant to report a digestive disorder which occurred on the two occasions when treatment A (dried grass and 4 lb. concentrates/gal.) followed treatment B (dried grass and 2 lb. concentrates/gal.). The symptoms in order of appearance were a scour, a refusal of concentrates, then of dried grass and silage, and finally complete lack of rumination. There was a reduction in food intake and consequently a decline in milk yield. When hay was offered it was consumed avidly, and when hay had been fed for a few days the cows regained their normal state of health. On these two occasions the ration for treatment C, the next treatment in the sequence, was fed, and a missing value calculated for the milk yield and food consumption on treatment A. On the six other occasions when treatment A was fed there was no trouble.

As far as the health of the udder was concerned as judged by cell counts and bacteriological examination, there were five quarters in two cows which had clinical mastitis for short periods and five quarters in three cows which developed severe subclinical mastititis. All ten quarters were treated with antibiotics. The attacks of clinical mastitis occurred in two cows on treatment B and were no doubt partly responsible for the lowered average lactose content in the milk on this treatment (Table 3).

Milk yield

The average milk yields per cow per day on each treatment are shown in Table 3. The treatments brought about substantial differences in milk yield, ranging from 31·8 lb./day

on treatment D to 38·7 lb./day on treatment A. The average daily milk yield of cows receiving concentrates at the rate of 4 lb./gal. (A and C) was 37·3 lb., and this was significantly greater ($P<0·01$) than the 33·5 lb. produced when they received concentrates at 2 lb./gal. (B and D). A similar increase in average yield (3·0 lb.) was observed when the cows received dried grass instead of hay (A compared with C, and B compared with D in Table 3), and this difference was also statistically significant ($P<0·01$). It is important to note that ration B (2 lb. concentrates/gal. and dried grass) and ration C (4 lb. concentrates/gal. and hay) produced substantially the same milk yield, in spite of the fact that on ration B the total dry matter eaten per cow per day was only 27·8 lb. compared with 32·0 lb. on ration C.

Table 3. *The mean daily milk yield and chemical composition of the milk*

		Milk composition (g./100 g. milk)				
Treatment	Milk yield (lb./cow/day)	Fat	Solids-not-fat	Crude protein	Casein	Lactose
A	38·7	3·91	8·59	3·15	2·45	4·49
B	35·1	4·05	8·45	3·12	2·42	4·39
C	35·9	3·93	8·51	3·15	2·44	4·42
D	31·8	4·08	8·42	2·98	2·33	4·48
Standard error of difference between two means						
	±0·87	±0·09	±0·05	±0·02	±0·03	±0·04

For each additional 1 lb. of concentrates fed per gallon of milk, the increases in milk yield were 2·3 and 2·0 lb. for the cows fed hay and dried grass, respectively. The residual effects of the treatments from one period to another were examined by the method of Williams(6) but nothing of statistical significance was found.

Milk composition

The weighted mean composition of the milk is shown in Table 3. Values for the two cows which had digestive trouble in the last 2 weeks of treatment B, were calculated from the results obtained in the second week of the period.

The changes in the fat and solids-not-fat content of the milk as a result of the four feeding treatments were for the most part small. Feeding concentrates at the rate of 4 lb./gal. resulted in a significant average increase of 0·11% S.N.F. over the 2 lb./gal. rate ($P<0·05$). On the other hand, no significant improvement in S.N.F. content was obtained when dried grass replaced hay in the rations, although this result is complicated by the mastitis which lowered the lactose content of milk produced on treatment B.

Cows on treatment D (2 lb. concentrates/gal. and hay) produced milk with a protein content significantly lower ($P<0·001$) than when they were on any of the other three treatments.

The fat content of the milk was slightly higher on the rations containing the smaller amounts of concentrates (B and D), but none of the differences in fat content between treatments was significant.

Live weights

The mean live weights of the cows on the four treatments are given in Table 4. None of the differences was significant.

The linear regression coefficient for each cow on each treatment was calculated. Very

few of the regressions were significant, and of the four pooled regressions for each treatment, only that for treatment D was significant ($P < 0{\cdot}001$). The regression coefficients are given in Table 4 and it can be seen that there was a gradation in live-weight change from −0·74 lb./day on treatment D to +0·34 lb./day on treatment A. The treatments affected milk yield and S.N.F. in a similar manner.

Table 4. *The mean live weights of the cows and the mean daily change during each feeding treatment*

Treatment	Mean live weight (lb.)	Change during feeding treatment (lb./day)
A	1072	+0·34
B	1059	−0·09
C	1073	0·00
D	1050	−0·74***
S.E. of difference between two means	±12	—

*** $P < 0{\cdot}001$.

DISCUSSION

In this experiment with rations which contained 40 lb. of good-quality grass silage per day, an increase in the rate of concentrate feeding from 2 to 4 lb./gal. resulted in the production of 3·8 lb. more milk per cow per day and an increase of 0·11% in the S.N.F. content. There was a fall of 0·15% in the fat content of the milk at the higher level of concentrate feeding, but this difference was not statistically significant. Similarly, the replacement of hay by dried grass in the ration increased the average milk yield by 3·0 lb./cow/day and the S.N.F. content by 0·05%, leaving the fat content unchanged. The common factor bringing about these changes in yield and S.N.F. content was, of course, the greater intake of nutrients resulting from either the higher level of concentrate feeding or the better quality grass product. There was only a slight increase in the consumption of silage, hay or dried grass, when the level of concentrate feeding was reduced from 4 to 2 lb./gal. of milk.

Nutrient values of rations in terms of starch equivalents and D.C.P. calculated from chemical analyses and feedingstuff tables are only very approximate, but they can give a useful indication of feeding level. The present results showed an increase of 0·9 lb. milk for each additional 1 lb. S.E. fed. This result was the same as that obtained in a previous experiment (11), but it should be noted that in both experiments an increase in S.E. intake was accompanied by a small increase in D.C.P. For example, an additional 3·8 lb. milk per day was produced in the present work when an extra 4·5 lb. S.E. and 0·8 lb. D.C.P. were fed.

Previous work on the value of dried grass as a substitute for concentrates was confirmed by the performance of the present cows on treatments B and C. Here concentrates fed at 2 lb./gal. plus dried grass per day (treatment B) were just as productive as 4 lb. concentrates/gal. plus hay per day (treatment C). Despite the probable inaccuracies in the calculation of nutrient intake it is interesting to note that the calculated amounts of S.E. and D.C.P. provided by these two rations were essentially the same.

Increasing consumption of energy and protein had only a small effect on milk composi-

tion. There were no significant differences between the fat contents of the milk produced on the four rations, and the only significant difference in S.N.F. contents was between ration D (hay and 2 lb. concentrates per gallon) and ration A (dried grass and 4 lb. concentrates per gallon). Mastititis probably lowered the S.N.F. content of the milk produced on ration B (protein normal, lactose abnormally low) and if this value is omitted the remaining values show a linear increase of 0·02% S.N.F. for each additional 1 lb. starch equivalent and 0·2 lb. D.C.P. fed above maintenance. This response of S.N.F. to extra feed is smaller than we have found previously, when values of 0·04–0·06% S.N.F. per 1 lb. S.E. have been recorded (11, 12). No adequate explanation can be given for the poorer response in the present work since the general composition of the diet, the amounts of fibre in it and the nutritional range of treatments were very similar to those in one of the previous trials (11). It may be that, as Burt (13) has noted, big changes in milk composition are usually associated with experiments in which some of the treatments involve very low levels of feeding, and in the present experiment even the lowest level of feeding was not below Woodman's standard in energy, the component most likely to alter S.N.F. contents.

The low level of S.N.F. values on all treatments was entirely the result of the lactose contents being about 0·2% lower than might be expected for Ayrshire cows at this stage of lactation (100 days calved) (9). Neither cell count nor bacteriological examination indicated that mastitis was responsible except where already noted. The increase in S.N.F. from the lowest level of feeding (ration D) was clearly shown to be the result of an increase in the protein fraction of the milk (Table 3).

The present results suggest that doubling the rate of concentrates fed per gallon to cows receiving hay and silage has only a small beneficial effect on milk S.N.F. Griffiths & Featherstone (14) recently reported the results of some trials in which 4 lb. maize (3 lb. S.E.) per day per cow was fed to fifteen cows on four farms as an additional supplement to rations already theoretically adequate. The average response was 0·03% S.N.F. per lb. increase in S.E. fed, a similar result to the present one. However, on two farms the milk S.N.F. of the supplemented cows increased by 0·18 and 0·06%, whilst on the other two it decreased by 0·02 and 0·10%. From the information given, the decreases in composition after extra feeding are not easily explained, although disease is always a possible factor.

When the margin of profit per day on the four treatments was compared, it was found that treatment D gave the highest margin, treatments B and C were intermediate and approximately equal and treatment A gave the lowest margin. Treatment D, however, resulted in a large loss of live weight and in these particular conditions, produced milk with a non-fatty solids content below 8·5%. Since this loss of live weight would have had to be made up at a later date and since there would probably have been a shortened lactation, it is unlikely that treatment D would have ultimately been the most profitable one. Treatment A resulted in a high level of nutrient intake and an appreciable gain in live weight and it would seem that this treatment was uneconomic for the milk yield achieved. The final choice of ration would, therefore, lie between either B, silage with dried grass + 2 lb./gal. concentrates, or C, silage with hay + 4 lb./gal. of concentrates. Both these treatments supplied similar intake of nutrients and had similar effects on milk yield, milk quality, live-weight changes and profitability. The experiment has again confirmed the value of high-quality grassland conservation products for dairy cattle feeding and the economy which they can make in the amount of concentrates fed.

SUMMARY

1. In a short-term winter feeding trial using eight Ayrshire cows, a concentrate with a nominal starch equivalent of 68 was fed at 2 lb. and 4 lb./gal. of milk with either 16 lb. hay or 16 lb. dried grass. Silage (40 lb. per day) was also fed.

2. The weight of dry matter from grass products eaten per day was similar on all four treatments. Differences in total daily dry-matter consumption were very largely attributable to differences in the consumption of concentrate dry matter.

3. The daily dry-matter consumption as a percentage of live weight increased from 2·5 on the rations that included hay and 2 lb. concentrates per gal. to 3·2 on the rations that included dried grass and 4 lb. concentrates per gal.

4. Milk yield was significantly increased from an average of 33·5 to 37·3 lb./day when the amount of concentrates fed increased from 2 to 4 lb./gal. and also significantly increased from 33·9 to 36·9 lb./day when dried grass was fed in place of hay.

5. There was an increase of 0·9 lb. milk for each 1 lb. increase of starch equivalent plus 0·2 lb. digestible crude protein intake as a result of either increasing the rate of concentrate feeding, or of feeding dried grass instead of hay.

6. The fat content of the milk was similar on all four rations, whereas the solids-not-fat varied from 8·42% on the ration that included hay + 2 lb. concentrates/gal. to 8·59% on the ration that included dried grass + 4 lb. concentrates/gal. This change in S.N.F. was almost entirely in the protein fraction of the milk.

7. There was a gradation in live-weight change from −0·74 lb./day on the hay + 2 lb. concentrates/gal. to +0·34 lb./day on the dried grass + 4 lb. concentrates/gal.

8. After a consideration of profit margins, milk quality and live-weight changes it was concluded that both treatments B (dried grass + 2 lb./gal.) and C (hay + 4 lb./gal.) gave optimum and almost identical results.

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724. LEVELS OF FEEDING OF CONCENTRATES FOR DAIRY HEIFERS BEFORE AND AFTER CALVING

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(With Figure 1)

The immediate effects on the milk yield of the dairy cow of concentrates fed above the accepted rate of approximately 4 lb. including 2·5 lb. starch equivalent, per 10 lb. milk, has been shown to be small (1, 2, 3, 4). However, a relatively large residual effect on lactation yield from a short period of high, or indeed low, level of feeding has been enunciated (5); and is illustrated in recent data from New Zealand (6, 7). Thus, from an economic point of view it is the long-term effect which is more important; the short-term effect may often be insufficient to cover the cost of extra food. High-level feeding in late pregnancy (steaming-up) has been advocated (5, 8) as a means of increasing milk yields. The practice has been adopted by many dairy farmers, although occasionally little benefit has been derived from it or the benefit has been largely confined to the first 3 months of lactation (9, 10).

During the past 3 years the effect of 'steaming-up' and of high-level feeding during early lactation have been studied singly and in combination in heifers at the National Institute for Research in Dairying. The effects, both immediately after calving and over the whole lactation have been studied.

EXPERIMENTAL METHOD

The animals used were the Shorthorn and Friesian heifers of the herd that calved during the autumn and early winter for the three seasons 1954–7. A 2×2 factorial design was used for the experiment, the treatments consisting of two levels of steaming-up and two levels of concentrates during the first 84 days of lactation. Animals were blocked according to expected date of calving and by breeds.

The two levels of steaming-up were:

High—beginning 21 days before the estimated date of calving, 4 lb. concentrates per day for 3 days, then 8 lb. per day for 4 days and finally 12 lb. per day until parturition.

Low—beginning 14 days before the estimated date of calving, 4 lb. concentrates per day until parturition.

The two levels of concentrates in lactation were:

High—5 lb. concentrates per 10 lb. milk.

Low—3 lb. concentrates per 10 lb. milk + 1 lb./day over the first 28 days of lactation. (These represent 125 and 75% of normal standards.)

The quantities were adjusted on an individual cow basis once per week. The concentrates used throughout were a proprietary mixture of cereals, cereal by-products, high protein oil cake, and minerals, and were made up into cubes. The components varied

a little from year to year but not within years. The ratio of energy to protein in the concentrates was maintained at that normally recommended for milk production.

The treatments will be referred to as HH, HL, LH and LL. The first letter of the combination indicates the level of steaming-up; the second indicates the level of lactation feeding.

Roughages were fed for maintenance. The heifers were rationed as a herd to grass in autumn and to kale during early winter. After Christmas they were fed individually weighed rations of 30 lb. lucerne silage and 8 lb. (Shorthorns) or 10 lb. (Friesians) of lucerne tripod hay for the years 1954–5 and 1955–6. In 1956–7 they were fed 50 lb. lucerne silage and 4 lb. meadow hay.

Concentrates were fed to individual cows by volume whilst the herd grazed but were weighed for the period after Christmas. Foods were sampled weekly for dry-matter estimation. These samples were bulked and subsampled for proximate analysis at the end of each season.

Steaming-up rations were fed before roughages in the morning. Half the daily concentrates ration was fed to heifers in milk before each milking and half the roughages after each milking. Milk yields were recorded at each milking as from the fourth day after calving. Samples of milk were taken at morning and evening milking 1 day per calendar month for the estimation of butterfat and solids-not-fat (S.N.F.) content.

Apart from the exceptions noted below, all heifers were treated alike from the 84th day of lactation. During the remainder of the winter they were fed concentrates at 4 lb. per 10 lb. milk plus hay and silage as above. During the spring and summer they grazed leys and received concentrates in proportion to yield at a rate appropriate to the quality of the grass and irrespective of previous levels of concentrates feeding. Two blocks of the Shorthorns and three blocks of the Friesians were used in separate short-term experiments of 42 days duration. No appreciable effects were recorded for milk yield and composition for the Friesians. Five Shorthorns showed a temporary increase in S.N.F. percentage in the milk. This has been ignored in the analysis of the lactational effects of the treatments in the present experiment.

Heifers were weighed twice per week before calving, on the second and third days after calving, and weekly thereafter. All weighings took place at 7.30 a.m.

Table 1. *Distribution of animals*

Year	Breed		Total
	Shorthorn	Friesian	
1954–55	24*	12	36
1955–56	28*	8	36
1956–57	—	16	16

* Including one Guernsey.

RESULTS

The numbers of animals which completed the full experiment are shown in Table 1. Data from twenty-two blocks were analysed, comprising thirteen blocks of Shorthorns and nine blocks of Friesians. Two Guernsey heifers, one on HL (1954–5) and one on LH (1955–6), were used to complete blocks of Shorthorns.

Food consumption

Precise details of food consumption for maintenance are not available for the full experimental period. Proximate analysis (Table 2) of silage and hay indicates an approximate intake of 6·1 lb. starch equivalent by Shorthorns and 6·8 lb. by Friesians during the later winter months. Autumn forage crops were not individually rationed, but it is considered that they supplied an adequate maintenance ration. Analysis of the batches of concentrates used are also given in Table 2. By accepted standards 4 lb. would supply

Table 2. *Proximate analysis of foods used in the experiment*

			Percentage analysis of dry matter						
Year	Food	% dry matter	Ash	Ether extract	Crude protein	Crude fibre	N-free extract	True protein	Calculated S.E. (11)
1954–55	Silage	21·6	10·9	2·8	12·0	35·5	38·8	9·5	11
	Hay	82·7	7·7	1·1	10·3	38·6	42·4	8·8	35
	Dairy cubes	85·1	8·3	4·3	23·2	8·7	55·6	19·9	65
1955–56	Silage	19·6	8·8	2·2	10·5	39·6	38·9	7·4	11
	Hay	82·9	9·9	1·4	13·7	34·5	40·5	10·6	35
	Dairy cubes	84·8	8·2	3·4	19·4	7·8	61·2	18·6	65
1956–57	Silage	23·0	8·5	3·1	12·9	29·5	46·0	9·1	11
	Hay	83·4	7·1	1·5	7·6	33·6	50·3	6·8	30
	Dairy cubes	84·5	7·7	3·5	21·6	7·3	60·0	20·3	65

adequate nutrients for 10 lb. milk. The mean consumption of concentrates for high and low levels of steaming-up were 244·5 and 53·5 lb. per cow. The actual quantities of concentrates fed for milk production during the first 84 days of lactation were, for the high level 0·51 lb. and the low level 0·31 lb./lb. milk produced. During steaming-up and the first 84 days of lactation, HH, HL and LH consumed respectively 827, 248 and 580 lb. per head more concentrates than LL (Table 4).

Milk yields

The mean daily milk yields for the various treatments are given in Table 3 for the 84 days of lactation and for the 305-day lactation. Fig. 1 shows the lactation curves for the first 84 days. During week 1 little difference occurred between the treatments, thereafter their effects were fully established. Over the 84-day period groups HH, HL and LH gave respectively 4, 3½ and 3½ lb. more milk per head daily than group LL,

Table 3. *Treatment mean daily milk yields in lb. and 305-day lactation yields*

					S.E. of difference of means	Steaming-up		Lactation feeding	
Period	HH	HL	LH	LL		High	Low	High	Low
Days 1–84	33·01*	32·37*	32·51*	29·04	1·60	32·69	30·78	32·77	30·70
Peak yield†	37·41*	36·38*	36·94*	33·38	2·08	36·89	35·16	37·17	34·88
Total yield days 1–84	2773*	2719*	2731*	2439	175	2746	2585	2757	2579
305-day (mean daily yield)	26·95*	26·79*	26·52*	22·13	1·10	26·87	24·32	26·73	24·46
305-day (total yield)	8220*	8171*	8089*	6750	634	8195	7419	8154	7460

* Significantly greater than LL at $P = 0{\cdot}05$.

† Measured as the mean daily yield for the 3 days over which the greatest amount of milk was produced.

(significant at $P = 0{\cdot}05$), HH showing little advantage over HL and LH. Over the 84-day period the extra milk per head from these treatments amounted to 334 lb. for HH, 280 lb. for HL and 292 lb. for LH. From the corresponding figures for total consumption of concentrates (steaming-up + lactation) (Table 4) it can be calculated that HH consumed 2·48 lb. concentrates per extra lb. milk, whilst HL and LH consumed 0·88 and 1·98 lb. concentrates, respectively. Thus, the extra milk produced on treatments HH and LH cost 1·60 and 1·10 lb. concentrates per lb. milk more than that produced on treatment HL at virtually the same overall output.

The milk yields over the 305-day period show that the treatments had a marked residual effect. Groups HH, HL and LH continued to outyield group LL throughout lactation.

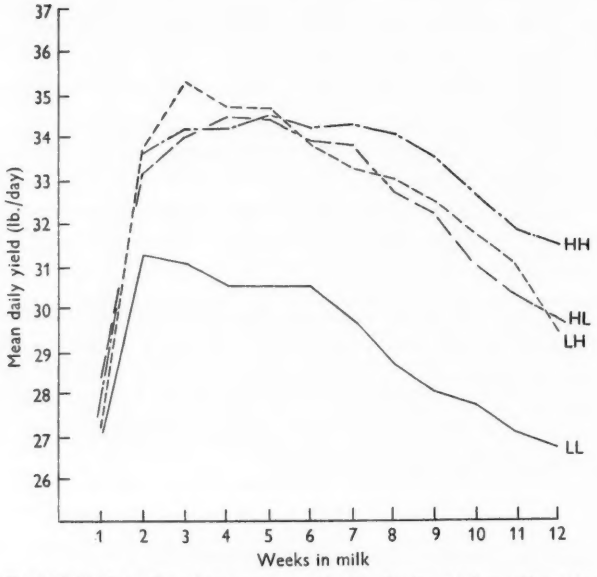

Fig. 1. Graph of milk yield (lb./day) of the four treatments during the first 84 days of lactation, based on weekly averages. — · — · —·, HH; — — — — —, HL; - - - - - LH; ————, LL.

Table 4. *Concentrate consumption per animal in lb.*

Treatments ...	HH	HL	LH	LL	Steaming-up High	Steaming-up Low	Lactation feeding High	Lactation feeding Low
Measurements								
Total steaming-up concentrates	248	239	50	57	243·5*	53·5*	—	—
Steaming-up + days 1–84 of lactation	1689	1110	1442	862	1399	1152	1565	986
Additional concentrates over group LL steaming-up + days 1–84 of lactation	827	248	580	—	—	—	—	—
Total concentrates/lb. milk, days 1–84 of lactation	0·61	0·41	0·53	0·35	0·51	0·44	0·57	0·38
Concentrates fed/lb. milk, days 1–84 of lactation	0·51	0·32	0·51	0·33	—	—	0·51	0·32

* Standard error of difference of means = 22·8 lb.

Their mean final milk totals were 1470, 1421 and 1339 lb., respectively, more than the 6750 lb. recorded for group LL (significant at $P=0{\cdot}05$). The advantage of approximately 30 gal. over the first 84 days was more than quadrupled over the lactation. The original outlay of concentrates was spread, therefore, over a greater additional yield.

The animals on the HH treatment reached their peak yield later (5–6 days) than animals on the HL and LH treatment, and these in turn were later (6 days) than animals on treatment LL (Table 5). In persistency, measured here as the recorded days

Table 5. *Time relationships in lactation (days)*

Treatment ...	HH	HL	LH	LL	S.E. of difference of mean	Steaming-up		Lactation feeding	
						High	Low	High	Low
Measurement									
Time to reach peak yield from fourth day after calving	31·5*	25·2	26·5	19·0	3·29	28·35	22·75	29·00	22·10
Persistency, i.e. time to reach half peak yield	296*	278	278	257	16·4	287	267	287	267

* Significantly greater than LL at $P=0{\cdot}05$.

in milk before yield fell to half peak yield, the difference of 39 days between HH and LL groups was significant. The intermediate value of 278 days for both HL and LH was non-significantly greater than the 257-day period recorded for group LL (Table 5). For both these measurements the values for HL and LH were almost midway between HH and LL. This suggests that the treatment effects were additive in this respect.

Milk composition

In this experiment steaming-up had no effect *per se* on butterfat percentage (Table 6). Since, however, milk yields were increased by the high level of steaming-up, butterfat yield was also increased. In contrast, level of lactation feeding had a significant effect

Table 6. *The percentages of butterfat and solids-not-fat in the milk, and the yields of butterfat and solids-not-fat for the various treatments*

Treatment ...	HH	HL	LH	LL	S.E. of difference of means	Steaming-up		Lactation feeding	
						High	Low	High	Low
Measurement									
Mean butterfat %, days 1–84	3·28	3·65*	3·30	3·56*	0·110	3·46	3·43	3·29	3·60
Mean butterfat %, 305-day lactation	3·38	3·69*	3·48	3·69*	0·108	3·53	3·58	3·43	3·69
Butterfat yield (lb./day), days 1–84	1·07	1·18	1·06	1·04	0·057	1·12	1·05	1·06	1·11
Butterfat yield (lb./day), 305-day lactation	0·902	0·988†	0·892	0·820	0·013	0·941	0·856	0·897	0·950
S.N.F. %, days 1–84	8·93	8·97	8·92	8·93	0·079	8·95	8·92	8·92	8·95
S.N.F. %, 305-day lactation	9·02	9·04	8·97	8·98	0·058	9·03	8·97	8·99	9·01
S.N.F. yield (lb./day), days 1–84	2·94	2·90	2·87	2·60	0·128	2·92	2·73	2·90	2·75
S.N.F. yield (lb./day), 305-day lactation	2·42	2·42	2·38	1·99	0·092	2·42	2·19	2·40	2·20
Mean yield of butterfat, days 1–84, corrected for milk yield variations (lb./day)	1·03	1·16	1·04	1·12	0·035	1·09	1·08	1·03	1·14
Mean yield of S.N.F., days 1–84, corrected for milk yield variation (lb./day)	2·83	2·85	2·81	2·82	0·024	2·84	2·81	2·82	2·83

* HL, LL significantly greater than HH, LH at $P=0{\cdot}05$.
† HL significantly greater than LL at $P=0{\cdot}01$.

on butterfat percentage. The high level of feeding produced higher yields of milk with a lower butterfat percentage (3·29%) than the low level of feeding (3·60%). This effect is not usual in itself. However, it is interesting that treatment HL gave a high yield of milk because of the high level of steaming-up and also produced milk of high butterfat percentage consistent with the general effect of the low level of lactation feeding. Consequently, the yield of butterfat from this treatment was higher than those from the other treatments. The difference between the butterfat yields for treatments HL and LL approached significance at $P = 0{\cdot}05$ for the 84-day period. The same trends continue for the 305-day lactation and group HL yielded a significantly greater weight of fat than group LL ($P = 0{\cdot}01$). Other differences between treatments were non-significant.

Both steaming-up and level of lactation feeding had no effect on the S.N.F. percentage in the milk (Table 6). Consequently, both for the first 84 days of lactation and for the 305-day period, the yields of S.N.F. mirrored the milk yields and those of groups HH, HL and LH were greater than that of group LL. By analysis of covariance it was shown that treatment effects on butterfat and S.N.F. yields were almost entirely due to milk yield, *per se* (Table 6).

Live weight

Table 7 gives a summary of changes in live weight over the lactation. The mean post-calving live weights for high and low levels of steaming-up were 1050 and 1047 lb.; these treatments had no appreciable effect on the gross live weight of the heifer herself at calving.

Table 7. *Details of live weight and changes in live weight for the four treatments*

Treatment ...	HH	HL	LH	LL
Measurement				
Live weight at calving	1048	1052	1065	1030
Live weight at week 6	1014	1016	1040	993
Live weight at week 12	1034	1026	1062	1017
Gain in live weight over lactation*	165	169	179	170†

* Based on sixty-seven animals and measured as the difference in post-calving live weight of first and second calving.

† Standard error of difference of means = 27 lb.

Analysis of the live-weight curves showed that the slope of the live-weight curve was not influenced by the level of steaming-up. However, the high level of early lactation feeding (HH and LH combined) reduced the fall in live weight after calving and increased the subsequent gain up to 12 weeks after parturition by $0{\cdot}757 \pm 0{\cdot}335$ lb./week (significant at $P = 0{\cdot}05$). On the other hand, the differences between treatments in live-weight change from first to second calving were non-significant. Data on only sixty-seven animals were available for the latter analysis; the remaining animals were discarded from the herd prior to second calving.

Table 8 sets out the principal measurements on milk yield and composition for treatments within breeds; no significant departures from the general result are apparent. The tendency for Friesians on LH to give higher milk yields than Friesians on HH and HL is notable, and suggests a greater response to improved nutrition in this breed. Also the similarity between all Friesian treatment groups in the time taken to reach half peak yield suggests that a strong inherent yield persistency exists in that breed.

Table 8. *Principal treatment mean values within breeds*

	Shorthorns					Friesians				
	HH	HL	LH	LL	Breed mean	HH	HL	LH	LL	Breed mean
Daily milk yield, days 1–84 (lb./day)	30·33	29·20	27·37	25·70	28·15	36·87	36·97	39·94	33·88	36·92
Butterfat %, days 1–84	3·29	3·73	3·39	3·51	3·48	3·26	3·53	3·16	3·65	3·40
S.N.F. %, days 1–84	8·92	9·02	8·97	8·84	8·94	8·94	8·90	8·86	9·06	8·94
305-day lactation yield (gal.)	704	692	642	537	644	941	996	1051	874	966
305-day butterfat (%)	3·39	3·77	3·58	3·69	3·61	3·36	3·57	3·35	3·68	3·49
305-day S.N.F. (%)	9·05	9·10	9·05	8·98	9·05	9·00	8·98	8·88	8·97	8·96
Presistency, i.e. days to reach half peak yield	288	255	256	221	255	306	310	309	307	308
Peak yield, mean of 3 days at maximum	34·43	33·37	31·33	30·06	32·30	41·72	40·73	45·04	38·12	41·41

Table 9. *Principal treatment mean values within years*

Year ...	1954–5				1955–6				1956–7			
Treatment ...	HH	HL	LH	LL	HH	HL	LH	LL	HH	HL	LH	LL
Daily milk yield, days 1–84 (lb./day)	32·84	34·60	32·48	29·60	32·38	28·96	31·10	27·65	34·79	35·07	35·74	30·93
Butterfat %, days 1–84	3·29	3·69	3·43	3·58	3·21	3·69	3·22	3·54	3·39	3·47	3·16	3·61
S.N.F. %, days 1–84	8·93	9·05	9·02	8·90	8·87	8·97	8·79	8·85	9·06	8·80	9·01	9·15
305-day lactation yield (gal.)	805	863	789	675	798	694	731	622	914	989	908	796
305-day butterfat (%)	3·28	3·61	3·51	3·74	3·44	3·86	3·44	3·63	3·48	3·51	3·52	3·71
305-day S.N.F. (%)	8·94	9·09	8·99	8·94	9·03	9·05	8·93	8·98	9·21	8·91	9·02	9·09
Time to reach peak yield (days)	21·4	22·1	24·7	11·8	43·6	25·7	30·1	24·3	27·6	31·5	21·2	23·2
Persistency, i.e. time to reach half peak yield (days)	296	277	266	246	292	263	285	248	299	311	285	297

In Table 9 the principal mean treatment effects are set out by years. Although the data for the individual years are too few for statistical analysis, they show that the main differences found when the results for the 3 years were combined, appeared in each year.

DISCUSSION

The feature of greatest interest is the effect of combinations of the alternative levels of steaming-up and of lactation feeding on milk yield and composition. Usually contrasting treatments before or after calving have been imposed on an otherwise constant plane of nutrition. Here it was found that steaming-up exerted an appreciable beneficial effect only when the level of feeding after calving was not above normal standards. Treatment HH produced only a little more milk than LH, whereas HL produced in all 140 gal. more than LL. Conversely, a generous preparation for lactation before calving drastically reduced the response to high-level feeding after calving, from 134 gal. (LH versus LL) to 5 gal. (HH versus HL).

Over the first 84 days the response to high-level lactation feeding after low-level steaming-up was 3·5 lb. milk for an extra 6·9 lb. concentrates which is approximately equivalent to 1 lb. milk for an extra 1 lb. starch equivalent. This rate of response is very similar to those reported by Burt (4).

The response in milk yield to treatments HH, HL and LH was cumulative over the lactation, in all cases the greater part of the additional milk being produced after the end of the actual experimental feeding period. This result confirms earlier work (5,6) and markedly influences the economic aspects of the treatments, to be discussed later.

Since treatments HH, HL and LH produced virtually the same increase in yield, treatment HH was extravagant in the use of concentrates, for a high level of feeding at either stage was equally as effective as a combination of the two in promoting milk yield.

The comparison of treatments HL and LH is technically and economically important, for the comparable levels of milk production are associated with greatly different efficiencies of utilization of concentrates. Over the 84-day period the additional quantity of concentrates consumed per additional lb. of milk produced by HL and LH were 0·88 and 1·98 lb., respectively. The total quantity of concentrates eaten by LH animals was 3 cwt. more than the quantity eaten by HL animals. It may be noted that the extra milk produced by HL resulted in an overall efficiency of conversion of concentrates which was only slightly less than that obtained with LL. The gross quantity of concentrates per lb. milk produced by the latter was 0·35 lb. and for the former it was 0·41 lb., during the first 84 days of lactation. It is not possible to reproduce this comparison for the full lactation because some milk was produced 'off the grass' during spring and summer, but it seems quite clear that high-level steaming-up increased milk yield at a lower food cost than high lactation feeding.

Table 10. *Comparison of the approximate costs of the additional milk produced by treatments HH, HL and LH*

	1–84 days			1–305 days		
	HH	HL	LH	HH	HL	LH
Extra milk (gal.)	33	28	29	147	142	134
Maximum estimated extra concentrates (lb.)*	827	248	580	1283*	704*	1036*
Return for milk†	£4. 19*s.* 0*d.*	£4. 4*s.* 0*d.*	£4. 7*s.* 0*d.*	£18. 7*s.* 6*d.*	£17. 15*s.* 0*d.*	£16. 15*s.* 0*d.*
Cost of extra concentrates‡	£10. 6*s.* 9*d.*	£3. 2*s.* 0*d.*	£7. 5*s.* 0*d.*	£16. 0*s.* 9*d.*	£8. 16*s.* 0*d.*	£12. 19*s.* 0*d.*
Surplus + or deficit − of return for milk over cost of concentrates	−£5. 7*s.* 9*d.*	+£1. 2*s.* 0*d.*	−£2. 18*s.* 0*d.*	+ £2. 6*s.* 9*d.*	+ £8. 19*s.* 0*d.*	+£3. 16*s.* 0*d.*

* Based on 4 lb. concentrates per gallon for all extra milk produced after the 84th day.
† At 3*s.*/gal. in winter and at 2*s.* 6*d.*/gal. over the year.
‡ At 3*d.*/lb.

In Table 10 an attempt has been made to compare the results of treatments HH, HL and LH in terms of cash. Costs and returns are necessarily arbitrary but serve to show the trends in the costs of the extra milk produced. Under the experimental conditions it was not, of course, possible to assess the overall costs of milk production. HL showed a small immediate surplus during the first 84 days of lactation and a very adequate one over the 305-day period. LH and HH actually showed an initial deficit which was converted to a small surplus over the lactation. A recent investigation in New Zealand (7) has shown similar effects from feeding concentrates for a short period to freshly calved cows at grass. In that experiment also a small immediate return was greatly increased over the lactation.

The higher fat percentage on the lower level of feeding of concentrates during the first 12 weeks of lactation (both HL and LL) undoubtedly merits further study, since it appears in all 3 years (Table 9) and was statistically significant for the 3 years combined (Table 6). There are several possible explanations for this, but none appears to be completely satisfactory. Thus, animals on HL treatment changed from a higher to a lower plane of nutrition at calving, and an effect on fat percentage comparable to that found by Eckles & Palmer (12, 13) may have occurred. This possibility is less likely with the LL

animals, although the precise relative level of nutrition before and after calving in this treatment is not easy to assess. The increase in butterfat percentage due to high steaming-up recorded on several occasions (5, 14, 15, 16, 17) cannot be deemed to have occurred here as the effect appeared in the LL treatment and not in the HH treatment.

A more attractive explanation lies in the theory that when milk yield is depressed and fat yield remains constant, the fat percentage is automatically increased. This explanation fits well so far as the LL treatment is concerned, but obviously not for the HL treatment since here the yield was as high as for the HH and LH treatments.

In the present experiment there is the added complication that during the first 12 weeks of lactation the HL and LL animals failed to recover weight to the same degree as the animals on the other two treatments. It seems possible that the interrelationship between milk yield, fat percentage and live-weight changes holds the secret of the substantially higher fat percentage of the animals that received the lower level of concentrate feeding after calving.

No effect was recorded here on the S.N.F. content of the milk of the heifers. On at least three occasions (1, 2, 19), the S.N.F. percentage in the milk of cows has been increased by extra feeding; Burt (3, 20), however, recorded a variable effect in a group of broadly similar experiments conducted at various centres.

There was in the present experiment a notable absence of a large effect on post calving live weight due to high steaming-up, such as has been observed elsewhere (6, 10, 17, 18). Here the treatments were not extreme, being neither so high in concentrates as the American experiments (10), nor so stringent in limitation of roughage as at least two of those reported from New Zealand (6, 17). The additional milk produced after high steaming-up was not correlated with additional elaboration of tissue in the heifer itself.

SUMMARY

1. Two levels of steaming-up and two levels of concentrates feeding during the first 84 days of lactation have been compared in a 2×2 factorial experiment over three winter seasons, using fifty-two Shorthorn and thirty-six Friesian heifers. The levels of steaming-up were 2 cwt. and $\frac{1}{2}$ cwt. of concentrates fed over the last 21 and 14 days of pregnancy, respectively. The levels of concentrates during lactation were 5 and 3 lb. per 10 lb. milk per day. Roughages were fed for maintenance. After the 84th day of lactation all animals were given the same treatment for the remainder of the lactation. Milk yield, milk composition and live weight were measured throughout the lactation.

2. The treatments were annotated as HH, HL, LH and LL, the first letter indicating level of steaming-up and the second the level of concentrates feeding in the first 12 weeks of lactation.

3. Mean milk yields were as follows:

	HH	HL	LH	LL
First 12 weeks of lactation (lb.)	2773	2719	2731	2439
305-day lactation (lb.)	8220	8171	8089	6750

4. The LH treatment required 3 cwt. more concentrates than HL to produce the same amount of milk.

5. The response to additional concentrates on the LH treatment after calving was 1 lb. additional milk per 1 lb. additional starch equivalent.

6. Butterfat percentages were higher (3·69%) in both groups on low-lactation feeding than in the two groups on high-lactation feeding (3·43%). The HL group (i.e. the group that received high steaming-up and low-lactation feeding) combined high milk yield with a higher butterfat percentage, and at current milk and feedingstuff prices and under the conditions of the trials, this group showed a greater cash return than the groups on the other three treatments.

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725. A COMPARISON OF DRIED SKIM-MILK AND WHITE FISH MEAL AS PROTEIN SUPPLEMENTS

III. FOR FATTENING PIGS FED WHEY WITHOUT RESTRICTION UNDER COMMERCIAL CONDITIONS

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(With 1 Figure)

Results obtained by Barber, Braude & Mitchell(1) and by Braude, Clarke, Mitchell, Cray, Franke & Sedgwick(2) in experiments with fattening pigs receiving all-meal diets, indicated that the protein supplement, white fish meal (10% reduced to 7% at 130 lb. live weight), could be replaced on a weight for weight basis by dried skim-milk without any adverse effect on the growth rate or efficiency of food conversion of the pigs. In the latter experiment, in fact, the pigs fed dried skim-milk grew rather better than the fish meal control animals, although their grading was adversely affected as a result of thicker back-fat measurements, particularly over the loin.

Braude, Clarke, Mitchell, Cray, Franke & Sedgwick(3) showed that reasonably satisfactory growth could be obtained by giving fattening pigs, receiving unrestricted amounts of whey, 3 lb./day/pig of a proprietary sow and weaner basal meal, containing approximately 17% crude protein, reduced to only 2 lb./day/pig by the time they were 13 weeks of age. The object of the experiment reported here was to obtain information on the relative value of dried skim-milk and white fish meal as protein supplements in the basal meal of pigs fed unrestricted amounts of whey.

EXPERIMENTAL

(1) *Treatments and diets*

All pigs received unrestricted amounts of whey. In addition they were given 3 lb./pig/day of a basal meal, fed dry, until they were 12 weeks (± 3 days) of age. The daily allowance of meal was then reduced over a period of one week to 2 lb./pig and continued at this level for the remainder of the fattening period. The composition of the four basal meals that were fed throughout the fattening period and which comprised the four experimental treatments is given in Table 1, together with their calculated crude-protein values.

The chemical composition of the dried skim-milk and white fish meal used is given in Table 2. Sufficient quantities of these two ingredients were obtained before the experiment began, to last for the whole trial, and were put through a mixer to ensure that they were homogeneous before taking samples for analysis.

It is seen from Table 1 that the total crude-protein content was similar for the basal

meals used on treatments 1 and 4, whereas in the basal meals given to the pigs on treatments 2 and 3, the total crude protein was lower corresponding to the amount of skim-milk included. In order that the total calcium and phosphorus content of the diets containing dried skim-milk should be approximately the same as that of the fish-meal diet, steamed bone flour was added to them. The amount of sodium chloride in the diets containing dried skim-milk was similar to or slightly higher than that in the fish-meal diet.

Table 1. *Percentage composition of basal meals used in the four treatments, together with the calculated crude protein values*

Treatment no. ...	1	2	3	4	
Fine millers' offal	50	50	47½	45	
Barley meal	40	38½	36½	34	
White fish meal	10	—	—	—	
Dried skim-milk	—	10	15	20	
Steamed bone flour	—	1½	1½	1	
Nuclo-P, No. 1A*	10 lb./2000 lb. of mix				in all diets
Aurofac 2A†	4 lb./2000 lb. of mix				in all diets
Crude protein (%)‡	18·3	15·4	16·6	17·8	

* From Cooper, McDougall and Robertson Ltd., and containing 800 i.u. vitamin A and 200 i.u. vitamin D_3/g.
† From Cyanamid of Great Britain Ltd., and containing 3·6 g. chlortetracycline/lb.
‡ Using the figures in Table 2 for the dried skim-milk and white fish meal, and figures given by Woodman(4) for the other ingredients.

Table 2. *Percentage chemical composition of the dried skim-milk and white fish meal used in the experiment*

	Dried skim-milk	White fish meal
Crude protein	36·8	65·3
Oil	0·3	3·6
Moisture	4·2	11·0
Ash	8·0	21·3
Calcium	1·28	6·44
Phosphorus	1·04	3·67
Sodium chloride	1·61	1·86

(2) *Pigs*

With the one exception given below, details of the pigs used, their pre-experimental management, breeds, selection for experiment and housing were all identical with those described in detail by Braude *et al.* (3). In the present experiment, however, the pigs used were weaned from their dams at 5 weeks ($\pm$3 days) of age on to Amvilac No. 1 pellets* fed dry, plus unrestricted amounts of whey. They continued to run on pasture in portable huts until 8 weeks ($\pm$3 days) of age, when they were selected for experiment and brought indoors.

The treatments were allocated at random within each replicate to the pens of nine group-fed pigs, as described in the previous experiment (Braude *et al.* (3)). There were five pens of pigs on each treatment, or a total of 180 pigs on the experiment.

The first two replicates of four pens of nine pigs each were selected on 26 May, the next two on 2 June and the fifth and final replicate on 9 June 1955.

(3) *Pre-experimental treatment of pigs*

For the first 3 days after being put in the experimental pens, all pigs continued to receive the Amvilac No. 1 pellets. A gradual change, over a period of 10 days, was then

* Glaxo Laboratories Ltd.

made to the four experimental meals, so that the experiment proper began when the age of the animals was 10 weeks (±3 days), that is, 2 weeks after the pigs had first been brought into the Danish-type fattening house.

(4) *Records*

All details of records kept, adjustment of food-consumption data to allow for pigs that either died or had to be taken off experiment for reasons discussed later, and method of conversion of whey consumed to a 12% moisture basis, were as described by Braude *et al.* (3), with the exception that as the pigs approached slaughter weight weighing was done twice, instead of once, weekly.

(5) *Carcass grading*

Each pig was sent to the bacon factory as a minimum live weight of 203 lb. was reached at the twice-weekly weighing. All other details of the slaughter arrangements and carcass measurements taken were as described by Braude *et al.* (3).

(6) *Biometrical methods*

Means for each variable were calculated for each pen and subjected to the usual analysis of variance for a randomized block design. In the analyses, adjustments were not made for variation in either initial live weight or cold dead weight (see Barber, Braude & Mitchell (5)).

(7) *Health of pigs*

During the course of the experiment, eleven out of the total of 180 pigs, or approximately 6%, either died or had to be taken off the experiment, the reasons for their removal being given in Table 3.

Table 3. *Details of pigs which died or were taken off experiment*

Treatment no.	No. of pigs involved	Cause of death, or removal from experiment
1	3	Paralysis hind legs and pneumonia. Broken leg. Rupture
2	1	Poor doer
3	2	Acute lameness. Poor doer
4	5	Two badly hurt fighting. Poor doer. Died enteritis and peritonitis. Prolapse of rectum

In spite of the fact that the fattening house was completely empty for 1½ weeks before the experimental pigs were put in the pens in an attempt to reduce the incidence of coughing characteristic of virus pneumonia which was known to exist in the herd (see Braude *et al.* (2, 3)), this disease was again fairly widespread amongst all the experimental pigs. The same measures to control the secondary complications of this pneumonia, as described by Braude *et al.* (3), were applied with a good degree of success. As an outbreak of parasitic mange had occurred in a number of pigs in the herd at the time when the experiment began, all experimental pigs were sprayed with benzene hexachloride as a precautionary measure. Subsequently, a few pigs showed symptoms of mange, but prompt further treatment with benzene hexachloride satisfactorily cleared it and none of the affected pigs suffered any setback in growth.

RESULTS

The mean results for live-weight gain, efficiency of food utilization and carcass measurements, together with the appropriate standard errors and levels of statistical significance are given in Table 4. The commercial grading results for all the pigs are summarized in Table 5, while in Table 6 the commercial grading results are subdivided according to sex. In Table 7, the pattern of food consumption over 14-day periods during the experiment is shown.

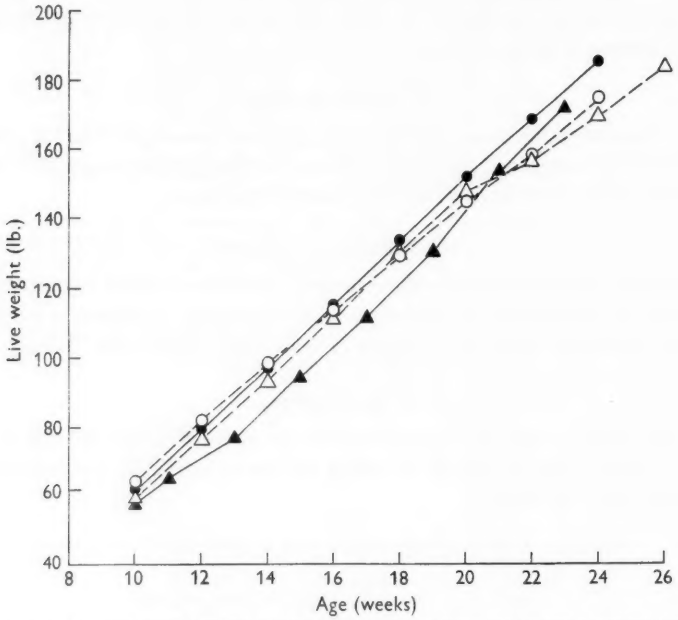

Fig. 1. Comparison of average growth curves of pigs in present experiment with those in two earlier experiments carried out under similar conditions. △ - - - △, present experiment. Average of 169 pigs. ○ - - - ○, Braude *et al.*(3). Average of 83 pigs given 3 lb. of meal/day reduced to 2 lb. by 13 weeks of age plus unrestricted amounts of whey (Exps. 1 and 2 combined). ●——●, Braude *et al.*(3). Average of 86 pigs given 3 lb. of meal/day throughout plus unrestricted amounts of whey (Exps. 1 and 2 combined). ▲——▲, Braude *et al.*(2). Average of 44 pigs given an all-meal diet in unrestricted amounts up to a daily maximum of 6½ lb./pig.

In Fig. 1, the average growth curve is shown of all the pigs on the experiment from 10 weeks of age up to the time when the first pig went to slaughter. The growth curves of some of the pigs on the two earlier experiments carried out on the Cow and Gate farm are also included (Braude *et al.*(2,3)).

(1) *Live-weight gain and efficiency of food utilization*

Replacement of the white fish meal by dried skim-milk, either on a weight for weight basis (10%), or at the level of 15%, had no significant effect either on the rate of growth or the efficiency of food utilization (meal + whey) of the pigs. Replacement of the white fish meal by dried skim-milk on a protein basis (20%), however, resulted in a significantly faster rate of growth ($P < 0{\cdot}05$) and a trend towards a more efficient utilization of the food.

Pigs receiving the 20% dried skim-milk basal meal also grew significantly faster ($P < 0.05$) and tended to utilize their food more efficiently than the pigs given the basal meal containing either 10 or 15% dried skim-milk.

Table 4. *Treatments, mean live-weight gain, efficiency of food utilization and carcass measurements of pigs during fattening period from approximately* 10 *weeks of age to a mean live weight of* 208 *lb. All pigs received* 3 *lb./day, reduced to* 2 *lb./day by* 13 *weeks of age, of a basal meal plus unrestricted amounts of whey*

Treatment no. ...	1	2	3	4	Differences between two means required for significance. Probability level		Standard error
Protein supplement in basal meal ...	White fish meal	Dried skim-milk					
	10%	10%	15%	20%	5%	1%	
No. of pigs	42	44	43	40	—	—	—
Daily gain (lb.)	1·10	1·11	1·11	1·16	0·044	0·062	0·014
Efficiency of food utilization:							
(*a*) lb. meal/lb. gain	1·95	1·95	1·93	1·85	—	—	—
(*b*) gal. whey/lb. gain	3·18	3·11	3·10	3·00	—	—	—
(*c*) lb. of 88% dry-matter food (meal + whey)/lb. gain	4·28	4·22	4·20	4·04	0·182*	0·255	0·059
Carcass length (mm.)	779·3	772·8	769·5	775·7	10·20*	—	3·31
Shoulder fat (mm.)	46·2	46·1	46·6	45·9	1·82*	—	0·590
Mid-back fat (mm.)	23·8	24·8	25·1	24·7	1·23*	1·73	0·399
Loin-fat (mm.) 1	33·3	34·2	33·4	33·2	1·50*	—	0·488
2	29·8	29·8	29·8	29·9	1·60*	—	0·520
3	36·0	36·3	36·7	36·5	2·14*	—	0·696
Belly thickness (mm.) 1	38·8	38·8	38·6	38·4	2·31*	—	0·749
2	35·3	36·0	34·7	35·1	2·08*	—	0·676
3	34·0	34·9	34·1	34·1	2·82*	—	0·916
Eye muscle (mm.) Depth	37·9	37·2	37·5	37·8	0·76*	—	0·246
Breadth	80·5	80·8	82·0	80·7	2·12*	—	0·687

* Mean square for treatment differences not significant at 5% level.

Table 5. *Commercial grading* results of pigs*

Treatment	No. of pigs	Final grade: Triple A No.	Triple A %	A No.	A %	B No.	B %	C No.	C %
1	42	17	40·5	33	78·6	9	21·4	0	0
2	44	18	40·9	33	75·0	7	15·9	4	9·1
3	43	18	41·9	33	76·7	7	16·3	3	7·0
4	40	16	40·0	30	75·0	7	17·5	3	7·5

* As in force at time pigs were slaughtered.

Table 6. *Commercial grading results. Comparison of male and female pigs*

Treatment	No. of pigs M.	No. of pigs F.	Triple A M.	Triple A F.	Final grade: A M.	A F.	B M.	B F.	C M.	C F.
1	21	21	6	11	16	17	5	4	0	0
2	21	23	6	12	14	19	4	3	3	1
3	21	22	6	12	13	20	6	1	2	1
4	20	20	7	9	13	17	5	2	2	1

Table 7. *Mean consumption of meal, whey and crude protein/pig/day over 14-day periods during the experiment*

Treatment ...		1			2			3			4		
Protein supplement in basal meal ...		White fish meal			Dried skim-milk								
		10%			10%			15%			20%		
Approximate age of pigs (weeks)	Approx. average live weight of all pigs in middle of each age period (lb.)	Meal (lb.)	Whey (gal.)	Crude protein (lb.)	Meal (lb.)	Whey (gal.)	Crude protein (lb.)	Meal (lb.)	Whey (gal.)	Crude protein (lb.)	Meal (lb.)	Whey (gal.)	Crude protein (lb.)
10–12	68	2·9	1·28	0·62	3·0	1·24	0·55	2·9	1·31	0·58	2·9	1·32	0·61
12–14	85	2·2	2·02	0·55	2·2	2·10	0·49	2·2	1·92	0·50	2·2	2·13	0·55
14–16	102	2·0	2·69	0·56	2·0	2·85	0·51	2·0	2·59	0·52	2·0	2·82	0·56
16–18	120	2·0	3·51	0·62	2·0	3·43	0·56	2·0	3·23	0·57	2·0	3·35	0·60
18–20	137	2·0	3·96	0·65	2·0	3·67	0·57	2·0	3·68	0·60	2·0	3·92	0·64
20–22	152	2·0	3·87	0·65	2·0	3·50	0·56	2·0	3·91	0·61	2·0	3·91	0·64
22–24	163	2·0	4·04	0·66	2·0	3·76	0·58	2·0	4·15	0·63	2·0	3·87	0·64
24–26*	176	2·0	4·25	0·67	2·0	4·30	0·62	2·0	4·42	0·65	2·0	4·70	0·70
26–28	—	2·0	4·81	0·71	2·0	4·61	0·64	2·0	4·75	0·67	2·0	4·94	0·71
28–30	—	2·0	4·67	0·70	2·0	4·70	0·65	2·0	4·41	0·65	2·0	4·99	0·72
30–32	—	2·0	4·08	0·66	2·0	4·37	0·62	2·0	3·77	0·60	2·0	4·34	0·67
32–34	—	2·0	3·20	0·60	2·0	4·77	0·65	2·0	4·49	0·66	—	—	—
34–36	—	2·0	3·60	0·63	2·0	4·45	0·63	—	—	—	—	—	—

* From this stage onwards pigs were sent for slaughter, consequently the figures refer to a continually changing number of animals.

(2) *Carcass measurements*

There were no statistically significant treatment differences in any of the carcass measurements made (Table 4).

(3) *Commercial grading results*

The similarity in the carcass measurements of all the pigs is reflected in the commercial grading results in Table 5. It is seen that the grading of the majority of the pigs in all the groups was quite satisfactory. However, whereas a few pigs from each of the three groups that received the dried skim-milk basal diets graded C, the control pigs all graded either A or B.

The superior grading of the female pigs compared with the males in each of the four groups is clearly shown in Table 6.

(4) *Pattern of food consumption during the experiment*

In considering Table 7, it must be remembered that some pigs from all treatments were ready for slaughter by the 24th–26th weeks of life, and that in consequence from this stage onwards the mean figures in the table refer to continually decreasing numbers of animals.

The results show that all groups of pigs attained their peak average daily consumption of about $4\frac{3}{4}$ gal. of whey/pig during approximately the 26th–28th weeks of life.

DISCUSSION

The results obtained in this experiment indicate that a protein supplement of 10% white fish meal can be replaced on a weight for weight basis by dried skim-milk in a basal meal fed with unrestricted amounts of whey, without adversely affecting the performance of

the pigs, and they thus substantiate the main findings of Barber *et al.* (1) and Braude *et al.* (2) in similar experiments with pigs on all-meal feeding.

In considering the relative value of the protein in white fish meal and in dried skim-milk, the two factors discussed in detail by Barber *et al.* (1), namely, that the level of protein in the control diet might be too generous and that the dried skim-milk diets had a higher energy value than the fish-meal control diet, must again be considered in the present experiment. In view of the relatively small amounts of the basal meal fed, however, one would expect the effect of both these factors to be much less than when all-meal diets are fed.

In Table 7 the approximate total daily crude-protein intake of the pigs over successive 14-day periods during the experiment are shown, account being taken of the protein in both the basal meal and whey in arriving at the figures. In Table 8 the corresponding

Table 8. *Average daily consumption of crude protein at different live weights of pigs receiving an all-meal diet containing 7% white fish meal to 90 lb. live weight and no protein supplement thereafter (obtained from data given by Woodman & Evans (6))*

Live weight (lb.)	Daily intake of crude protein (lb.)
60	0·50
70	0·59
80	0·67
90	0·72
100	0·57
120	0·66
140	0·74
160	0·80
180	0·83
200	0·87

figures are given for the pigs in the experiment of Woodman & Evans (6) which received a meal diet containing 7% white fish meal to 90 lb. live weight and no protein supplement thereafter. The results of the trials carried out by these authors indicated that the protein intake of the pigs on this type of diet was approaching the minimum required for optimal performance. A strict comparison between the protein intake figures according to live weight, given in Tables 7 and 8, is not possible, since in the former experiments all the pigs were group fed and each of the average live-weight figures given covers quite a wide range, whereas in the latter, individually fed pigs were included. Moreover, the precise minimum protein requirements of fattening pigs under different conditions has still to be determined. Nevertheless, the data in the two tables do suggest that the pigs on treatment 1 in the present experiment, receiving the basal meal with 10% white fish meal, were unlikely to have received protein greatly in excess of requirements. The results of this experiment, together with the findings of Barber *et al.* (1) and Braude *et al.* (2), therefore, strongly suggest that as a protein supplement, dried skim-milk is of greater value to the fattening pig than white fish meal, when compared on a protein content basis.

It is of interest that the general performance of the pigs receiving the 10% white fish-meal basal mixture plus unrestricted amounts of whey on treatment 1, agrees very closely with that of the pigs receiving similar treatment in the two trials reported earlier by Braude *et al.* (3), which were carried out in the same piggeries. Such close agreement between the two experiments is very satisfactory from an experimental point of view and adds weight to the significance of the results obtained.

It is seen from the growth curves in Fig. 1 that in both the present experiment and in the earlier trials (Braude *et al.* (3)) in which the pigs received the same meal and whey feeding (3 lb. meal reduced to 2 lb. meal/pig/day by 13 weeks plus unrestricted amounts of whey), there was a falling off in the rate of growth as the pigs got older. In the present experiment this reduction in the rate of growth was particularly marked from about the 20th week of life.

The pigs in the earlier trials reported by Braude *et al.* (3), which received 3 lb. meal/day plus unrestricted amounts of whey throughout the fattening period, also showed some falling off in the rate of growth, although it was less marked, from about the same age. In contrast, it is seen from Fig. 1 that the pigs in the experiment reported by Braude *et al.* (2) which received an all-meal diet, tended to show a gradual increase in the rate of growth throughout the fattening period.

These results indicate that in the later stages of the fattening period the pigs, restricted to only 2 lb. meal/day, are unable to consume sufficient whey to enable them to maintain at least a constant rate of growth, and that it might be advantageous to increase the daily meal allowance by $\frac{1}{2}$–1 lb. when the pigs are about 20 weeks of age. However, the possible adverse effect of raising the level of feeding in this way on carcass grading would need to be carefully examined.

The reasonably satisfactory grading results of all the pigs in the present experiment confirm the findings of Braude *et al.* (3) that under the conditions prevailing in these experiments, the feeding of 3 lb. meal, reduced to 2 lb. meal/pig/day by 13 weeks of age, with unrestricted amounts of whey, produces reasonable bacon carcasses.

It is of interest that whereas in the experiment of Braude *et al.* (2) with all-meal feeding the replacement of white fish meal by dried skim-milk in the ration had a marked adverse effect on grading results, in the present trial with whey feeding, the differences in the grading results of the two groups of pigs were very much less. This was undoubtedly due to the restriction of the basal meal to only 2 lb./pig/day for the greater part of the fattening period in the present experiment.

The age at which the pigs reached their peak average daily consumption of whey, namely, during the 26th–28th weeks of life, agrees approximately with the results of the second of the two trials reported by Braude *et al.* (3), but not with the first of these trials, where the peak intake occurred some 6–8 weeks earlier. Both the present trial and the second of the earlier trials mentioned above were carried out during the summer, whereas the first was run during the winter, and it is possible, therefore, that these differences in age at which peak whey consumption was reached, were at least in part attributable to seasonal effects. One would, however, have expected the reverse effect, namely, peak consumption of whey at an earlier age during warmer weather, and moreover a further complication in the interpretation of these different results arises from the fact that the magnitude of the peak whey consumption (approximately $4\frac{3}{4}$ gal./pig/day) in the present experiment agrees more closely with that obtained in the first, than with that obtained in the second of the two trials reported by Braude *et al.* (3). In the latter the average daily peak intake of whey of the pigs which received 2 lb. of meal/day from 13 weeks of age was only about $3\frac{3}{4}$–4 gal./pig. It is clear, therefore, that further work is required before the real significance of these differences in the pattern of whey consumption can be assessed.

SUMMARY

1. An experiment has been carried out under commercial conditions to compare the value of dried skim-milk and white fish meal as protein supplements in a basal meal fed to fattening pigs in conjunction with unrestricted amounts of whey.

2. There were four treatments. All pigs received 3 lb./pig/day of a basal meal, reduced to 2 lb. by the 13th week of life, plus unrestricted amounts of whey. The protein supplement in the four basal meals comprising the four treatments were: (1) 10% white fish meal, (2) 10% dried skim-milk, (3) 15% dried skim-milk and (4) 20% dried skim-milk.

There were five pens of nine group-fed pigs on each treatment, involving a total of 180 pigs. The pigs were on experiment from approximately 10 weeks of age to bacon weight. Comprehensive carcass measurements were made on all the pigs.

3. The results showed that the white fish meal in the basal meal fed with unrestricted amounts of whey could be replaced by dried skim-milk on a weight for weight basis (10%) without adversely affecting the performance of the pigs. Increasing the percentage of dried skim-milk to 15% appeared to have no advantage, but increasing it to 20% resulted in a slightly better performance of the pigs.

4. The results are discussed in relation to the findings in previous experiments and it was concluded that the evidence obtained so far strongly suggests that as a protein supplement, dried skim-milk is superior to white fish meal for the fattening pig, when compared on a protein content basis.

5. The commercial grading results of the pigs on all four treatments were quite good, with no very marked differences between any of the groups.

6. An average daily peak consumption of about $4\frac{3}{4}$ gal. whey/pig was reached by all groups of pigs during about the 26th–28th weeks of life. The growth curve and pattern of food consumption of the pigs during the experiment were compared with those in two earlier trials carried out under similar conditions, and possible reasons for some of the differences observed were discussed.

We thank the Directors of Messrs C. and G. Prideaux Ltd., Bacon Curers, Motcombe, Shaftesbury, Dorset, for permission to take carcass measurements on all experimental pigs, and we are greatly indebted to Mr K. Lawrence, who most conscientiously took these measurements. We are also most grateful to Miss J. N. King for her help with the statistical computations.

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726. HISTOLOGICAL CHANGES IN THE MAMMARY GLAND OF GUINEA-PIGS DURING LACTATION

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(With 1 Plate and 1 Figure)

INTRODUCTION

The importance of studying the changes in the mammary gland during lactation with reference to the mechanism of lactational performance has been indicated in our earlier study on the relation between milk production and mammary structure in dairy goats (1). There are, however, but few studies on this problem apart from our own, for example, those by Turner & Schultze (2) on rats, and by Turner & Gomez (3, 4) on mice and guinea-pigs.

This work was carried out to study the histological changes in the mammary gland of guinea-pigs during lactation, and also the relationship between histological factors and the deoxyribonucleic acid (DNA) content of the gland in order to ascertain the feasibility of using the DNA content as a possible basis for expressing the metabolic activity in the lactating mammary gland.

EXPERIMENTAL

Material. The material was obtained from the animals described in the previous report (5) on the lactose synthesis rate of mammary slices *in vitro*, and killed at the following stages: day 0 (day of parturition), day 2 or 3 (at peak of lactation), day 8 (during declining phase of lactation) and day 15 (just prior to weaning). Each group contained five animals. The mammary glands were dissected off, weighed and the lateral portions of both glands were used for histological study (see previous paper, (5)).

Histological methods. Four blocks from the two glands of each animal were fixed in Zenker formol (Helly) for 1 day, rinsed in tap water and embedded in collodion–paraffin, the procedure being as described in the report of Cowie, Folley, Malpress & Richardson (6). Sections were cut at 10 μ, and every fortieth section was mounted by the method of Richardson (7) and stained with Ehrlich's haematoxylin and eosin.

The porosity index (defined as the alveolar surface area per unit volume of tissue, i.e. $2n/l$, where n is the number of intersections made by a grid of length l with the alveolar epithelium, see Cowie *et al.* (6), Richardson (8) and Benson, Cowie, Cox, Flux & Folley (9)) was obtained using a grid of length 10 cm. The number of alveoli per projected field of diameter 10 cm (magnification $\times 248$) was also obtained in 200 fields (ten fields in five sections from each of four blocks) chosen systematically as recommended by Eränko (10). Further, the modal and the largest alveolar diameters were selected subjectively (see Benson, Cowie, Cox & Golzveig (11)) and measured using the ocular micro-

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meter; the modal and the largest heights of the glandular cells were likewise obtained. The numbers of glandular cells in the modal and the largest alveoli were counted and the number of glandular cells per field, i.e. per constant area, was calculated by multiplying number of cells in the modal alveolus by number of alveoli per field (C × E in Table 1).

Estimation of DNA. The procedure was described in the previous report (5), being mainly based on the method of Schneider (12) for extraction, and that of Dische (13) for estimation. The DNA contents were expressed on the dried-weight basis of the tissue (D.W.).

Statistical treatment. The number of fields used for the determination of the porosity index and for counting the number of alveoli as described above, was taken as 200 as a result of the analysis of variance in two animals on the variance due to blocks, sections and fields (Snedecor (14)). For all groups the means and the standard errors of the means were calculated. Differences between groups were examined by the '*t*' test after testing the homogeneity of populations by the '*F*' test. Where the populations of groups differed, significance of differences was tested using $(n_1 - 1)$ degrees of freedom, instead of $(n_1 + n_2 - 2)$, as recommended by Snedecor (14). To establish relationships between factors within the same stage of lactation, coefficients of correlation were calculated. The significance of these were tested using Fisher's table of '*t*' (Fisher & Yates (15)).

RESULTS

Results are shown in Table 1. The porosity index and number of alveoli per field were highest at parturition, lowest at the peak of lactation, but rising again from the decline of lactation to the time of weaning with significant differences within the 1% level. Modal alveolar diameter, and number of cells per modal alveolus showed an opposite trend

Table 1. *DNA content and histometric results*

	A	B	C	D	E	F
Stage (day of lactation)	No. of animals	Mean porosity index (cm.$^{-1}$)	Mean no. of alveoli per field	Mean diameter of modal alveolus (μ)	Mean no. of cells per modal alveolus	Mean no. of cells per field (C × E)
I (day 0)	5	106·9 ± 0·43	82·1 ± 0·33	40·4 ± 2·43	20·4 ± 1·29	1637 ± 60·8
II (day 2 or 3)	5	80·9 ± 0·37	47·2 ± 0·21	60·8 ± 1·45	26·6 ± 1·21	1244 ± 16·9
III (day 8)	5	90·0 ± 0·42	52·0 ± 0·22	56·3 ± 0·96	25·0 ± 0·63	1296 ± 33·2
IV (day 15)	5	95·5 ± 0·42	56·7 ± 0·24	51·3 ± 0·75	24·8 ± 0·58	1404 ± 16·7

	A	G	H	I	J	K
Stage (day of lactation)	No. of animals	Mean height of modal cell	DNA per mg. D.W.	Diameter of largest alveolus (μ) (range within group)	No. of cells in largest alveolus (range within group)	Height of tallest cell (μ) (range within group)
I (day 0)	5	5·8 ± 0·25	43·7 ± 2·23	66·0 ~ 110·3	37 ~ 53	10·3 ~ 14·4
II (day 2 or 3)	5	7·4 ± 0·21	30·1 ± 0·65	105·2 ~ 144·3	51 ~ 84	14·4 ~ 15·5
III (day 8)	5	9·1 ± 0·21	30·6 ± 1·73	116·5 ~ 141·2	58 ~ 68	15·5 ~ 16·5
IV (day 15)	5	11·1 ± 0·21	36·9 ± 0·91	111·3 ~ 117·5	56 ~ 62	18·6 ~ 19·6

(see also, Pl. 1), but in the latter the differences between the later stages of lactation (stages II and III, II and IV, and III and IV, respectively) were not significant. Cell heights, both modal and largest, increase as lactation progresses.

The number of cells per field, i.e. per constant area, and the DNA content of the dry matter showed a trend similar to that shown by the porosity index, and by the number of alveoli per field.

DISCUSSION

The results of this work agree well with our previous findings in goats (1). In both cases, the number of alveoli per unit area of the gland and the height of cells were low at the peak period and increased thereafter. The alveolar diameter and the number of cells per alveolus were largest at the peak period and decreased thereafter, even though the size of the modal alveolus was studied in the present work, whereas in the former report it was the mean alveolar diameter which was considered.

In order to investigate the relationships between the histological factors the coefficients of correlation were calculated and are shown in Table 2. The porosity index has a close relationship with the number of alveoli per field and is negatively correlated with the modal alveolar diameter. The porosity index is a very convenient factor for expressing the structural condition of the gland, but these correlations show that it is not always suitable to use by itself; for example, when its value is small it cannot differentiate small and scattered alveoli from large but closely packed alveoli.

Table 2. *Coefficients of correlation*

Combination	Stage of lactation I	II	III	IV
Porosity index:				
No. of alveoli per field	0·970**	0·985**	0·899*	0·842
Diameter of modal alveoli	−0·922*	−0·909*	−0·971**	−0·818
No. of cells in modal alveoli	−0·932*	−0·884*	−0·952*	−0·681
No. of alveoli per field:				
Diameter of modal alveoli	−0·982**	−0·964**	−0·920*	−0·995**
No. of cells in modal alveoli	−0·962**	−0·948*	−0·786	−0·878
No. of cells per field	0·947*	0·399	0·808	0·013
No. of cells per field DNA/mg. D.W.	0·951*	0·966**	0·967**	0·914*

* $0{\cdot}05 > P > 0{\cdot}01$. ** $0{\cdot}01 > P$.

The number of cells per field seems to be greatly influenced by the number of alveoli per field (i.e. the alveolar density), at certain stages (stage I and III), but as there is the negative correlation between number of alveoli per field and (*a*) the size of the alveolus (expressed as the diameter) and (*b*) the number of cells per modal alveolus, this influence is not always marked at other stages (stage II and IV).

In general, the coefficients of correlation are rather lower at stage IV, and this seems to be caused by the shrinkage of the alveoli. There are hardly any differences in the number of cells per modal alveolus between the stages after the peak period, therefore this shrinkage is caused by the alveolar cells becoming more slender.

Relationships between these histological factors and the DNA content were also investigated. At all stages there was a high positive correlation between the number of cells per field and the DNA content per mg. dry weight (see Table 2 and Fig. 1). Assuming

that the relationship between the dry weight (D.W.) and the sectional area of the gland remains constant throughout the different stages, and that the number of cells per field obtained by multiplying the number of cells per modal alveolus by the number of alveoli per field is statistically reliable, then since there is a straight line relationship between the number of cells per field and the DNA content per mg. dry weight, it may be said that

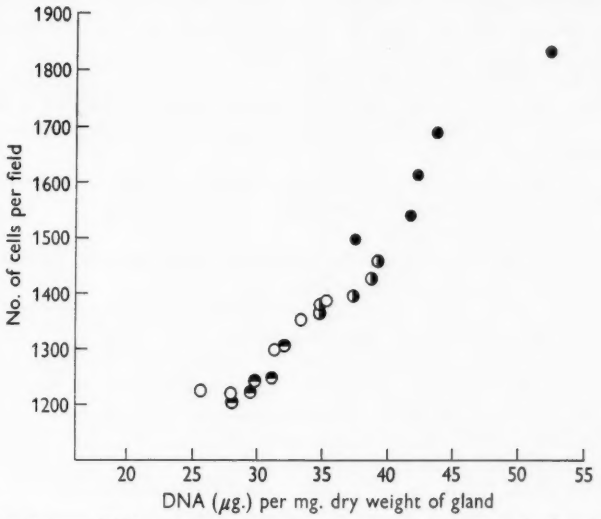

Fig. 1. Relationship between DNA content of mammary tissue and the number of cells per microscopic field (diameter 0·4032 mm). Lactation: stage I = ●, stage II = ◓, stage III = ○, stage IV = ◑.

this is in agreement with the view that the DNA content per cell remains constant. The relationship between DNA content and the number of cells has been demonstrated by Harkness & Harkness (16), Griffith & Turner (17) and Damm & Turner (18) in the developing mammary gland, and the present findings suggest, that in lactation, the DNA content may also be a good basis for expressing the metabolic activity of the gland as suggested by other workers (19, 20, 21, 22, 23, 5).

SUMMARY

Certain histological factors of the mammary gland and the DNA content unit per dry weight were studied at four stages of lactation in guinea-pigs.

The porosity index, the number of alveoli per field, the number of cells per field and the DNA content, showed maximal values at parturition and minimal values at the peak of lactation, whereas the opposite trend was shown by the value for modal alveolar diameter and the number of cells per modal alveolus.

The relationships between these results are discussed.

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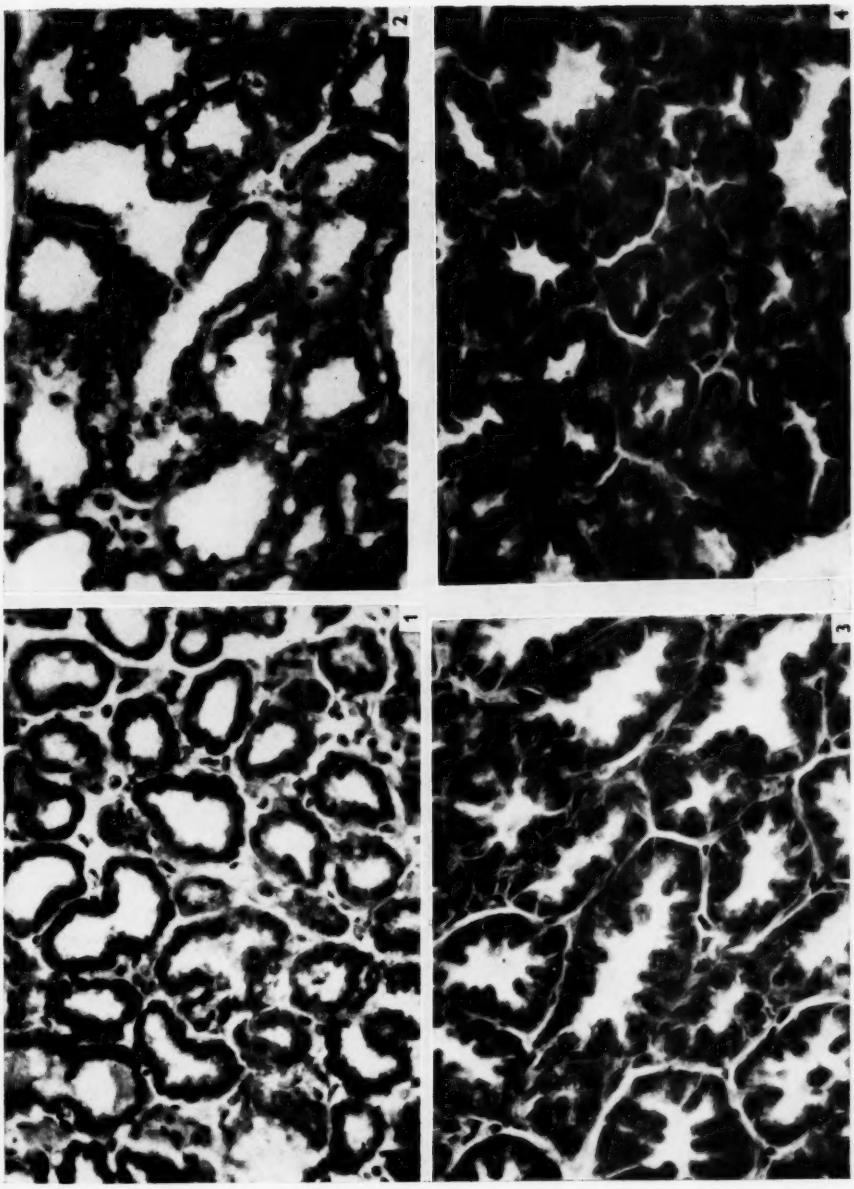

Microscopic appearance of the alveoli at the various stages of lactation (all at magnification of ×325). Stage I. Alveoli are small, glandular cells remain compact. Stage II. Alveoli are large, glandular cells remain compact. Stage III. Alveoli have decreased in size, glandular cells are taller. Stage IV. Alveoli are much decreased in size and glandular cells have become slender.

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727. THE VARIATIONS IN THE *PER CAPITA* CONSUMPTION OF MILK BETWEEN TOWNS IN GREAT BRITAIN, AND THEIR CAUSES

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That there are variations in the *per capita* consumption of milk between families has been amply demonstrated—the reports of the National Food Survey, conducted by the Ministry of Agriculture, Fisheries and Food, record them year by year (1). Some, at least, of the reasons for these variations—such as result from differences in income and in the size and composition of families—are also known. There are, in addition, variations in the *per capita* consumption of milk between different towns and areas of Britain, and it is the purpose of this paper to consider them.

A survey into the household consumption of milk in sixteen towns was conducted by the Agricultural Economics Research Institute, Oxford, in 1938, and this gave some information on this subject (2). A study made by the Statistics Department of the Milk Marketing Board, also at that time, but of a somewhat different character, provided further material relating to seventy towns. Both these studies relating to the immediate pre-war periods showed that the *per capita* consumption of milk was, as might be expected, especially low in the towns with low incomes and high unemployment. The, then, Ministry of Food made an investigation into the regional variations in the consumption of food in 1949. At that time the main foods were rationed, and, in consequence, it might be expected that regional variations in food consumption would be narrow. This investigation showed, however, that the consumption of milk was lower in north-eastern England than elsewhere (3). The controls over the quantities of milk that consumers could buy came to an end in 1950 and a more recent survey, made for the Milk Marketing Board in nine towns in 1955, indicated that substantial variations in the consumption of milk between towns still existed and that, out of this small sample of nine, it was especially low in two north-eastern towns—Leeds and Newcastle (4).

A further and more comprehensive source of information on this subject—as, indeed, it is on the consumption of all foods—is the National Food Survey. This survey has as one of its sampling criteria sixty parliamentary constituencies (reduced to fifty in 1956) which are changed with a new sample from year to year. Data upon the household consumption of milk was thus obtained from this source relating to the sample of sixty constituencies in two periods, April 1953 to December 1954 and the whole of 1955—120 constituencies in all—together with ancillary information, such as upon family income, size and composition in these constituencies, necessary in attempting to afford explanations of such variations in the consumption of milk as appeared. Descriptions of the sampling methods and of the techniques of the National Food Survey are given as appendices to the Annual Reports of the National Food Survey Committee (1). Of particular value in this respect is the account given with the Committee's Annual Report of 1953.

The average household consumption of milk among the sixty constituencies included in the National Food Survey in 1953–54 was 4·60 pints per person per week with thirty-two constituencies above and twenty-eight below this average. The corresponding figures in 1955 were an average of 4·62 pints per person per week with thirty-four constituencies above and twenty-six below this level. A frequency distribution of the constituencies according to their *per capita* milk consumption is given in Table 1, showing the 2 years

Table 1. *Frequency distribution of parliamentary constituencies according to their* per capita *consumption of milk at home*

Per capita milk consumption as percentage of the mean	No. of constituencies		
	1953–54	1955	Total
115 and over	1	3	4
110–114·9	8	8	16
105–109·9	18	13	31
100–104·9	8	10	18
95–99·9	7	8	15
90–94·9	8	8	16
85–89·9	3	4	7
80–84·9	4	4	8
75–79·9	2	1	3
70–74·9	1	1	2
Total	60	60	120

'Low' constituencies with *per capita* milk consumption 10% or more below the mean

1953–54	%	1955	%
Coatbridge (Scotland)	−10	Stirling and Falkirk (Scotland)	−10
Motherwell (Scotland)	−11	Barkston Ash (Yorkshire)	−10
Pontypool (Monmouth)	−15	Leeds, north-west (Yorkshire)	−11
Huddersfield (Yorkshire)	−18	Newcastle on Tyne, east (Durham)	−12
Hemsworth (Yorkshire)	−19	Don Valley (Yorkshire)	−13
Gateshead (Durham)	−21	Pontypridd (Glamorganshire)	−14
Houghton (Durham)	−25	Bishop Auckland (Durham)	−15
Dewsbury (Yorkshire)	−26	Rhondda, west (Glamorganshire)	−18
Sunderland (Durham)	−30	Brighouse and Spenborough (Yorkshire)	−19
		Consett (Durham)	−25
		Barnsley (Yorkshire)	−27

separately and together. This frequency distribution is skewed with the greater number of constituencies at or slightly above the average level and a 'tail' of constituencies having a relatively low milk consumption. In other words, there appear to be a number of constituencies with a low usage of milk, but few with an exceptionally high level above the usual or most frequent tendency. These 'low' constituencies—named below the table —are mainly situated in north-eastern England.

Since 1939, the *per capita* consumption of milk in England and Wales has risen substantially. The pre-war level was 3·0 pints per week compared with 4·9 pints in 1956. At the same time the variations between different towns or constituencies would also appear to have narrowed. Evidence of this is given in Table 2, in which a comparison is made between the results of the two pre-war investigations on a town basis to which reference has already been made and those from the National Food Survey on Parliamentary Constituencies. The average level of milk consumption shown by the National Food Survey in 1953–54 and 1955 was well above that found in the pre-war studies, and the variations about it, both in absolute and relative terms (when measured by the standard deviation and coefficient of variation, respectively), were smaller. With a general

increase in the consumption of milk, a more uniform pattern would thus appear to have developed throughout the country. It would seem, therefore, that certain of the factors that were the cause of such variations before the war have since diminished in importance.

Table 2. *The variations in the* per capita *consumption of milk between towns or parliamentary constituencies before and since the war*

Survey	No. of towns or constituencies	*Per capita* milk consumption per week: Mean (pints)	Standard deviation (pints)	Coefficient of variation (%)
Pre-war				
(*a*) 1936 Milk Marketing Board Study	70	2·14	0·84	38
(*b*) 1938 Agricultural Economics Research Institute	16	3·41	0·79	23
Post-war				
(*a*) 1953–54 National Food Survey	60	4·60	0·51	11
(*b*) 1955 National Food Survey	60	4·62	0·44	10

Variations in the retail prices of milk between different towns or parliamentary constituencies cannot afford an explanation of their varying levels of milk consumption. Since the war, these have been fixed by the Government and are standard throughout the country and, prior to the war, there was little variation in them: the main differences then arose between town and country areas rather than between towns. Variations in the retail prices of milk can thus be discounted as an explanation of differing levels of milk consumption between towns.

Differences in the average level of family income, on the other hand, appear to have been an important factor in the situation before the war. In the milk consumption survey made by the Agricultural Economics Research Institute in 1938, information was also collected on family food expenditure. This was obtained to be used as a measure of family income since it is more readily provided at interviews with participating housewives. Family food expenditure, when used as a measure of income, understates, however, any progressive upward change in income: as income rises between families, food expenditure rises, but less than proportionately. A simple correlation analysis was made between the average *per capita* milk consumption and food expenditure given for the various towns as the result of this survey. The results showed a high correlation between them (correlation coefficient, $r = 0{\cdot}76$), indicating that the variations in food expenditure between the towns could account for about half of the variations in *per capita* milk consumption. This would suggest a higher degree of correlation between income and *per capita* milk consumption should information on income have been available. In the study made by the Milk Marketing Board Statistics Department in 1936, estimates of *per capita* milk consumption in thirty out of the total of seventy towns were related to a measure of income. The resulting correlation coefficient ($r = 0{\cdot}80$) was again high, suggesting that differences in *per capita* income between towns could account for about two-thirds of the variations in *per capita* milk consumption.

It would thus appear that variations in income were, pre-war, one of the more important factors accounting for variations in the *per capita* milk consumption between towns. To judge from the results of the National Food Survey in more recent years, on the other hand, this situation has since changed. A correlation analysis between *per capita* milk

consumption and food expenditure in the various parliamentary constituencies in 1953–54, to be comparable with that derived from the survey results of the Agricultural Economics Research Institute in 1938, gave a low correlation coefficient of no statistical significance. A correlation analysis made between *per capita* milk consumption and income in the various parliamentary constituencies in both 1953–54 and 1955 also gave a low but, this time, a significant correlation ($r = 0{\cdot}40$), suggesting that variations in income between the constituencies could explain but about 15% of the variations in *per capita* milk consumption between them.

One reason for the smaller variations in the *per capita* consumption of milk between towns now as compared with before the war is, therefore, that income is not such an important factor in the situation. The variations in income between different towns and areas are not as great as they were then and unemployment, one of the main causes of income variations, is virtually non-existent.

Table 3. *Variations in the* per capita *consumption of 'all milk' between towns or parliamentary constituencies before and since the war*

	Per capita milk consumption per week				
	Mean			'All milk'	
Survey	Fresh milk (pints)	Condensed, evaporated or dried milk* (pints)	Total 'all milk' (pints)	Standard deviation (pints)	Coefficient of variation (%)
Pre-war					
(*a*) 1938 Agricultural Economics Research Institute	3·41	0·44	3·85	0·63	16
Post-war					
(*a*) 1953–54 National Food Survey	4·60	0·26	4·86	0·49	10
(*b*) 1955 National Food Survey	4·62	0·27	4·89	0·46	9

* In fresh milk equivalent.

Associated with the increases in fresh milk consumption since 1939, there have been changes in the use of condensed and evaporated milk. In the years immediately prior to the war, the *per capita* consumption of condensed and evaporated milk (including skimmed, condensed milk) in the United Kingdom amounted to 13·1 lb. per annum, but in 1955 it was 6·3 lb., less than half the pre-war figure (5). Information was also collected on the consumption of these preserved milks in the Agricultural Economics Research Institute Survey in 1938. This is given in Table 3, together with corresponding information from the National Food Survey in 1953–54 and 1955. The table shows that the variations in the consumption of 'all milk' (i.e. including both fresh and preserved milk) between towns was less pre-war than the variations in the consumption of fresh milk alone (a coefficient of variation of 16% as compared with 23% as shown in Table 2). This suggests that in those areas where the consumption of fresh milk was low, there was a tendency for it to be made good by preserved milk. The results of the National Food Survey, on the other hand, indicate that in recent years allowances for preserved milks make little difference to the inter-town variations: the coefficients of variation of fresh milk and of 'all milk' are much the same. This change is related to the changed importance of income in this situation. Prior to the war, income was an important factor affecting the consumption of fresh milk, and where it was low the consumption of the cheaper, preserved milks—especially

sweetened, skimmed, condensed milk—was high. Since that time, with changes in the level and distribution of incomes, fresh milk has been used in place of the preserved milks in the hitherto less favoured sections of the community; hence the variations between towns in their consumption of milk have narrowed.

The changes in the variations in the consumption of milk between different towns—now as compared with pre-war—have been largely associated with changes in the level and distribution of incomes and with the changes in the incidence of unemployment. Variations in the consumption of milk still, however, exist, and it was noted earlier that there are a number of towns (or parliamentary constituencies) where the household consumption of milk is unusually low. The questions then arise as to whether these variations may be the result of the income levels or of the size of the families in the areas concerned, since these are two known factors affecting milk consumption, or whether certain local tastes, habits or social factors may be involved. There may, for example, be certain local or regional food consumption habits resulting in a low consumption of milk in particular areas which remain after allowance has been made for their levels of income and their average family size.

Estimates were thus made of the general relationships between the household consumption of milk and family income and size in the samples of constituencies included in the National Food Survey in the 2 years 1953–54 and 1955. The 'double-logarithmic' form was used and, on the basis of these relationships, the 'expected' household consumption of milk in the various towns was calculated according to their family income and size. These were then compared with the actual household consumption of milk in the respective towns and the differences expressed as a percentage of them. These differences indicate the extent to which factors other than family income and size have influenced the consumption of milk in them and also include, of course, any residual errors. With certain qualifications, they give a measure of the effect of local habits and tastes upon the consumption of milk. The most important of these qualifications is that these so-called local factors also influence the regression equations taking account of family income and size. The direction in which this influence operated can, however, be indicated. The form of the relationships between average family milk consumption and average family income and size in the various constituencies in the 2 years was:

$$1953\text{–}54 \quad \log Y = 0{\cdot}77 + 0{\cdot}23 \log X_1 + 0{\cdot}33 \log X_2,$$
$$1955 \quad \log Y = 0{\cdot}57 + 0{\cdot}35 \log X_1 + 0{\cdot}45 \log X_2,$$

where Y = average household consumption of milk (in pints per week),
X_1 = average total family income (in £'s per week); and
X_2 = average number of persons in a family.

These relationships indicate that the income elasticity of the demand for milk, when using data on a parliamentary constituency basis, was of the order of 0·3. Using individual family, rather than constituency, data from the National Food Survey the income elasticity is rather less than 0·2, being even lower in the smaller families. The results from this family data are also influenced by local or regional habits and tastes, but in view of the reduced weighting put upon them this influence is reduced. It thus appears that the effect of these local or regional habits and tastes is such that the influence of income as shown by the constituency analysis is overstated. Where constituency income tends to be low, the habit and taste factor giving a low consumption of milk tends to be high.

In other words, the differences between the 'expected' consumption of milk derived from the above formulae and the actual consumption of milk, tend to understate the influence of local habits and tastes where these result in a low consumption of milk, and vice versa.

A complete list of constituencies, showing their actual and 'expected' consumption of milk is given in Appendix I to this paper, and Table 4 shows those constituencies where the total household consumption of milk was 10% or more above or below the 'expected' level derived from the regression estimates. Bearing in mind the qualification mentioned

Table 4. *Percentage deviation of household milk consumption from 'expected' level—high and low constituencies*

Parliamentary constituency	Percentage below 'expected' level	Parliamentary constituency	Percentage above 'expected' level
Dewsbury (Yorkshire)	−28	Caernarvon	+21
Barnsley (Yorkshire)	−25	South Angus	+19
Sunderland, south (Durham)	−24	Totnes (Devon)	+16
Brighouse and Spenborough (Yorkshire)	−22	Reigate (Surrey)	+15
		North Devon	+15
Consett (Durham)	−21	Maldon (Essex)	+14
Houghton-le-Spring (Durham)	−19	Hertford (Hertfordshire)	+14
Gateshead, west (Durham)	−18	West Dorset	+14
Pontypool (Monmouth)	−17	South Fylde (Lancashire)	+12
Hemsworth (Yorkshire)	−15	Portsmouth Langstone (Hants)	+12
Rhondda (Glamorgan)	−15	Eastbourne (Sussex)	+12
Leeds, north-west (Yorkshire)	−14	Abingdon (Berkshire)	+12
Holland with Boston (Lincs)	−14	Croydon, east (Surrey)	+12
Pontypridd (Glamorgan)	−13	Petersfield (Hampshire)	+11
Newcastle on Tyne (Durham)	−10	Northwich (Cheshire)	+10
Bishop Auckland (Durham)	−10	Cirencester and Tewkesbury (Gloucester)	+10
Grimsby (Lincoln)	−10		

above, the 'low' towns are of more interest than those with a consumption of milk above that which would be 'expected' from their levels of income and family size. It might be noted, however, that these 'high' constituencies are, in general, situated either in the south of England, or in agricultural areas in which the level of the consumption of milk is influenced by farm households and by the cheap or free supplies of farm workers employed upon dairy farms. The location of the 'low' towns where, according to these estimates, there is a low inherent demand for milk, is noteworthy. They are almost without exception in north-eastern England or in South Wales, and they form in the main the 'tail' of towns in the frequency distribution given earlier. It would thus appear that certain local variations in the inherent demand for fresh milk exist and that these form a regional pattern in which north-eastern England, generally, and parts of South Wales stand out as areas where it is low.

Various reasons can be suggested as to why this condition exists in these areas. They were, in general, areas especially affected by depressed economic conditions in the 1930's and where unemployment was then severe. At that time, considerable quantities of sweetened, skimmed, condensed milk was used in them and little fresh milk. It is possible that with improvements in prosperity since that time adjustments in food consumption habits and in the consumption of milk have not been made such as to fully match the changes in income. Heavy industry is also a feature of these areas and the type of work involved, and the associated social habits and conditions may also be a factor. It may

be noted that *per capita* food expenditure in these areas was slightly above the general level, suggesting that the position regarding milk did not extend to food as a whole.

From this study of the variations in the consumption of milk between towns (or parliamentary constituencies) in Great Britain, the following conclusions may thus be drawn:

(1) Variations in the *per capita* consumption of milk between different towns exist, ranging in a sample of 120 parliamentary constituencies from four constituencies at 15% or more above to 20 constituencies at 15% or more below the average level.

(2) These variations have, however, narrowed since before the war in both absolute and relative terms. In relation to the average level of milk consumption these variations are about a half of what they were before the war.

(3) This narrowing in the variations of *per capita* milk consumption has to a large extent resulted from a changed importance of income as a factor determining milk consumption, following upon a more even distribution of income between towns and a reduction in unemployment which, pre-war, especially affected certain towns and areas. Prior to the war, the variations in family income between towns accounted for over half and, possibly, two-thirds of the variations between them in *per capita* milk consumption. In more recent years they have accounted for but a minor proportion of the variations in milk consumption. Coupled with the changes in income and the influence of income upon fresh milk consumption, the use of preserved milk—notably sweetened, skimmed, condensed milk—has declined and has been replaced by fresh milk.

(4) Since the importance of income as a factor causing variations in the consumption of milk has declined, other factors such as local habits and tastes have become of greater relative importance, and the narrower but remaining variations largely result from them.

(5) The frequency distribution of towns (or parliamentary constituencies) according to their *per capita* milk consumption is skewed with few towns above the common tendency, but with a 'tail' of towns with a low consumption of milk.

(6) The low *per capita* consumption of milk in this 'tail' of towns cannot be entirely explained in terms of their average family income and size. It would thus appear to be connected with certain local habits or taste. The demand schedule for milk in the towns with a relatively low *per capita* consumption, at least in relation to income, is different from, and at a lower level than, elsewhere. Inherent local or regional variations in the demand for milk would thus seem to exist.

(7) These towns with a low consumption of milk are situated mainly in north-eastern England and in South Wales. Their low inherent demand for milk may remain as a carry-over from the impoverished conditions they, in general, suffered during the depression of the 1930's. It may, also or alternatively, be associated with the type of employment (usually heavy industry) in these towns, or from the social habits connected with particular types of work.

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Appendix I

(Actual and 'expected' household consumption of milk in parliamentary constituencies included in the National Food Survey, 1953–54 and 1955).

1955	Actual milk consumption per week per family (pints)	'Expected' milk consumption per week per family (pints)	Difference (pints)	Percentage difference	1953–54	Actual milk consumption per week per family (pints)	'Expected' milk consumption per week per family (pints)	Difference (pints)	Percentage difference
Wales									
Pontypridd	12·7	14·5	−1·8	−13	Caernarvon	17·0	14·0	+3·0	+21
Rhondda	12·5	14·7	−2·2	15	Pontypool	11·8	14·2	−2·4	−17
Conway	14·5	14·5	—	—	Barry	13·8	14·8	−1·0	− 7
Scotland									
Edinburgh, south	16·9	16·2	+0·7	+ 5	Glasgow, Hillhead	14·4	14·3	+0·1	+ 1
West Dumbartonshire	15·2	14·1	+1·1	+ 8	Central Ayrshire	15·2	15·4	−0·2	− 1
South Angus	15·9	13·4	+2·5	+19	West Fife	16·7	15·5	+1·2	+ 8
Stirling and Falkirk	13·9	14·7	−0·8	− 5	Aberdeen, north	16·1	14·9	+1·2	+ 9
Glasgow, Maryhill	16·5	16·0	+0·5	+ 3	Coatbridge and Airdrie	15·2	15·5	−0·3	− 2
Midlothian and Peebles	16·5	15·8	+0·7	+ 5	Motherwell	15·3	15·5	−0·2	− 1
North, East and West Ridings									
Leeds, north-west	12·6	14·7	−2·1	−14	Sunderland, south	11·8	15·5	−3·7	−24
Kingston upon Hill, north	13·2	13·9	−0·7	− 5	Dewsbury	10·2	14·1	−3·9	−28
Barnsley	11·4	15·1	−3·7	−25	Sheffield, Brightside	14·3	15·0	−0·7	− 5
Don Valley	14·2	14·9	−0·7	− 5	Houghton-le-Spring	12·2	15·1	−2·9	−19
Bishop Auckland	12·5	13·8	−1·3	−10	Hemsworth	12·9	15·2	−2·3	−15
Newcastle on Tyne, east	13·5	14·9	−1·4	−10	Doncaster	13·3	14·6	−1·3	− 9
Brighouse and Spenborough	10·8	13·9	−3·1	−22	Huddersfield, east	11·1	14·2	−3·1	−22
Consett	11·7	14·9	−3·2	−21	Gateshead, west	12·2	14·9	−2·7	−18
Barkston Ash	13·4	14·3	−0·9	− 6	Hexham	14·6	15·0	−0·4	− 3
North-west									
Birkenhead	16·8	15·6	+1·2	+ 8	Cheadle	15·1	14·4	+0·7	+ 5
Bury and Radcliffe	12·1	13·6	−1·5	−11	Manchester, Blackley	14·9	15·0	−0·1	− 1
Manchester, Cheetham	13·6	13·3	+0·3	+ 2	Accrington	12·6	13·7	−1·1	− 8
Northwich	15·7	14·4	+1·3	+10	City of Chester	15·2	14·7	+0·5	+ 3
Altrincham and Sale	14·4	14·9	−0·5	− 3	Liverpool, Walton	15·3	15·1	+0·2	+ 1
Liverpool, Toxteth	14·7	14·5	+0·2	+ 1	Salford	14·0	14·7	−0·7	− 5
Ince	13·4	14·3	−0·9	− 7	Leigh	14·2	15·2	−1·0	− 6
Lancaster	15·1	14·4	+0·7	+ 5	South Fylde	16·6	14·9	+1·7	+12
North-midland and east									
Leicester, south-east	14·8	14·4	+0·4	+ 3	Nottingham Central	12·8	14·1	−1·3	−10
Ipswich	13·4	13·9	−0·5	− 4	Peterborough	14·7	14·9	−0·2	− 1
Holland with Boston	12·6	14·6	−2·0	−14	Maldon	16·3	14·4	+1·9	+14
Hitchin	15·9	15·3	+0·6	+ 4	S.W. Norfolk	15·6	14·6	+1·0	+ 7
Billericay	15·5	14·6	+0·9	+ 6	Hornchurch	16·9	15·8	+1·1	+ 7
N.E. Derbyshire	14·5	15·2	−0·7	− 5	Grimsby	13·3	14·8	−1·5	−10
Bassetlaw	15·0	15·0	—	—	Hertford	17·0	14·9	+2·1	+14
Wellingborough	13·9	13·5	+0·4	+ 3	Harborough	15·9	14·9	+1·0	+ 6
Midland									
Birmingham, Sparkbrook	15·6	15·3	+0·3	+ 2	Oldbury and Halesowen	15·6	15·3	+0·3	+ 2
Bilston	15·1	15·3	−0·2	− 1	Rowley Regis and Tipton	14·1	14·9	−0·8	− 6
Leek	15·6	14·8	+0·8	+ 6	Newcastle under Lyme	15·4	14·6	+0·8	+ 6
Solihull	17·5	16·1	+1·4	+ 9	Birmingham, Northfield	16·7	15·6	+1·1	+ 7
Worcester	15·3	14·9	+0·4	+ 3	Hereford	15·5	14·9	+0·6	+ 4
South-western									
Plymouth, Sutton	15·9	14·9	+1·0	+ 7	Bristol, west	13·5	13·6	−0·1	− 1
Yeovil	14·4	13·8	+0·6	+ 5	Totnes	15·9	13·7	+2·2	+16
Cirencester and Tewkesbury	15·9	14·4	+1·5	+10	Weston-super-Mare	15·0	14·2	+0·8	+ 6
North Devon	15·2	13·2	+2·0	+15	West Gloucester	15·7	15·1	+0·6	+ 4
South-east and Greater London									
Edmonton	16·0	15·3	+0·7	+ 5	Hendon, south	14·9	14·9	—	—
St Pancras, north	15·7	15·0	+0·7	+ 4	Lewisham, north	15·9	14·7	+1·2	+ 9
Lewisham, south	14·1	13·1	+1·0	+ 8	Southwark	14·1	14·4	−0·3	− 2
Lambeth Norwood	16·5	15·9	+0·6	+ 4	Brentford and Chiswick	14·3	14·3	—	—
Harrow, central	15·3	15·6	−0·3	− 2	Uxbridge	16·1	15·2	+0·9	+ 6
Kensington, north	15·0	15·0	—	—	Paddington, north	14·4	13·8	+0·6	+ 4
Bermondsey	14·2	14·4	−0·2	− 2	Hammersmith, north	15·8	14·9	+0·9	+ 7
East Surrey	17·8	16·3	+1·5	+ 9	Kingston upon Thames	15·8	14·9	+0·9	+ 5
West Ham, south	14·1	14·9	−0·8	− 6	Leyton	15·2	14·5	+0·7	+ 4
Croydon, east	16·1	14·4	+1·7	+12	Bexley	17·1	16·8	+0·3	+ 1
South and south-east									
Eton and Slough	15·7	15·4	+0·3	+ 2	Portsmouth, Langstone	16·4	14·7	+1·7	+12
Petersfield	15·8	14·2	+1·6	+11	Canterbury	14·8	14·2	+0·6	+ 5
Maidstone	16·4	15·4	+1·0	+ 7	Basingstoke	16·7	17·9	−1·2	− 7
Brighton, Kemptown	13·4	13·3	+0·1	+ 1	Poole	14·8	14·1	+0·7	+ 5
West Dorset	15·4	13·6	+1·8	+14	Eastbourne	15·2	13·6	+1·6	+12
Gravesend	14·4	14·8	−0·4	− 3	Reigate	17·1	14·9	+2·2	+15
Chertsey	16·2	15·1	+1·1	+ 7	Abingdon	17·1	15·3	+1·8	+12

Appendix II

(Parliamentary constituencies with a high or low household consumption of milk.)

This chart shows the location of the various parliamentary constituencies according to the percentage by which the household consumption of milk in them was above or below that expected from their average family income and size. The data for each constituency is given in Appendix I. The constituencies where the actual consumption of milk was low in relation to what might be expected from their income levels and average family sizes were situated mainly in north-eastern England and in south Wales—heavy industrial areas.

Key: ●, constituencies with milk consumption 5% or more above that 'expected'. ○, constituencies with milk consumption less than plus and minus 5% of that 'expected'. ⊖, constituencies with milk consumption 5% or more below that 'expected'.

637.331.4:576.8

728. LACTOBACILLI IN THE AIR OF CREAMERIES

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Lactobacilli occur in large numbers in cheese (1), even when the milk used for cheese-making has been pasteurized (2) and therefore contains very few or no lactobacilli. The lactobacilli in cheese must enter during cheese-making, and a potential source of these organisms is the air of the creamery. Olson & Hammer (3), although not concerned directly with lactobacilli, showed that the numbers of bacteria in the air of dairy plants fluctuated with weather conditions. Naylor & Sharpe (4) found lactobacilli in the air of the Experimental Dairy at the National Institute for Research in Dairying: some strains were identical with others isolated from cheese, but there was frequently no correlation in type of lactobacilli from the air with those from cheese. Thus, 'cheese types' were found in the air some months before or after the cheese was made, which seemed to indicate contamination of the air from sources within the dairy. To obtain further information regarding the lactobacillus flora of the air the following investigation was made.

Air samples were taken in the cheese-making rooms at seven creameries and at three stages of processing at one processed cheese factory in Somerset, Wiltshire and Dorset. A slit-sampler (5) was used, organisms being collected on 2% plain agar, over which a layer of selective acetate agar medium (6) was poured. Plates were incubated in an atmosphere of 90% H_2 + 10% CO_2 for 5 days at 30° C.; representative colonies were then picked and isolates identified by means of cultural tests (7, 8, 9).

Table 1. *Numbers and species of lactobacilli isolated from the air of the cheese-making rooms at seven commercial creameries*

Creamery	Colony counts: slit-sampler machine run at 1 cu.ft./min. for 10 min.	30 min.	Species isolated
A	11	24	*L. acidophilus*, *L. plantarum*
B	210	~850	*L. casei*
C	55	38	*L. casei*
D	7	31	*L. casei*
E	14	71	*L. casei*, *L. brevis*
F	91	>2000	*L. casei*
G	55	297	*L. casei*, *L. brevis*, *L. plantarum*

Colony counts ranged from <1/cu.ft. to >65/cu.ft. (Table 1), though in the two creameries giving the extreme values, the same species, *Lactobacillus casei*, was isolated. The relatively high counts obtained at the processed cheese factory (Table 2), although the floors remain dry throughout the factory and all products are kept in air-tight packs,

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are probably due to the fact that mature cheese, known to contain lactobacilli in large numbers, is used as the raw material. For the remaining seven factories the colony counts broadly reflected the general conditions of the cheese-making rooms. The cheese-room at creamery C had been sprayed with hypochlorite solution immediately before the air was sampled. The low 30 min. count (compared with the 10 min. count) may therefore be due to inactivation of organisms by the disinfectant, since the 30 min. sample was taken after the 10 min. sample.

Table 2. *Numbers and species of lactobacilli isolated from the air at a processed cheese factory*

Colony counts*			
1	2	3	Species isolated
~770	31	72	*L. casei*, *L. plantarum*

* The slit-sampler was run at 1 cu.ft./min. for 15 min. in each case. (1) Sample taken close to homogenizing machine. (2) Sample taken in room used for cutting normal Cheddar cheese into 10 oz. portions and vacuum-packing them. (3) Sample taken close to machine wrapping and packing homogenized cheese in 1 oz. portions.

The single occurrence of *L. acidophilus*, although interesting, is of no importance in cheese-making, as this species will not grow at the temperatures used for ripening (about 15° C.). Homofermentative strains greatly outnumbered heterofermentative strains amongst the species isolated, *L. casei* being found in seven creameries and *L. plantarum* in three, whereas *L. brevis* was isolated only twice. The predominance of *L. casei* is not surprising, since the habitat of this organism is milk; *L. plantarum* and *L. brevis*, on the other hand, are of plant origin (10).

The lactobacillus species most commonly found in cheese are thus shown to be present in the air of cheese factories, and are a probable source of these organisms in the cheese. These same three species have recently also been found in starter cultures (11): it seems likely that such contaminants may well have come originally from the air.

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729. CHARACTERISTICS OF A PRESUMPTIVE PEDIOCOCCUS OCCURRING IN NEW ZEALAND CHEDDAR CHEESE

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Sherwood(1) investigated the bacterial flora of some thirty-three pasteurized-milk Cheddar cheeses and isolated and identified the dominant organisms as strains of *Lactobacillus casei* and *L. plantarum*. Strains of *L. brevis* and of *Leuconostoc* species were found to occur in small numbers also. During a study of the flora of ripening New Zealand Cheddar cheese, commenced in 1951, over 4000 cultures of these lactic acid-producing bacteria were isolated. The majority of them were the typical rod-shaped species of lactobacilli as described by Sherwood(1). However, many of the isolates from all the twenty cheeses examined consisted of large cocci with a predominant and characteristic diplococcoid form, and were initially thought to be strains of *Leuconostoc*. These cocci appeared to be part of the normal bacterial flora of cheese and were further investigated. Since they are unlike any previously described lactic acid-producing bacteria occurring in Cheddar cheese, including the *Leuconostoc* species, the cultural characteristics and biochemical behaviour of a representative culture designated A2 are now described.

MATERIALS AND METHODS

Cheeses. All the cheeses, from which samples were regularly taken during the ripening process, were manufactured from good quality flash-pasteurized milk, using either a single- or mixed-strain starter culture of *Streptococcus cremoris*. Fifteen of the cheeses were made in the Institute's cheese factory, while another five cheeses were obtained from commercial cheese factories in the North Island of New Zealand. The cheeses were stored at 55° F. for the first 2 weeks and were then waxed and stored in a cheese-curing room held at 45° F. and 80 to 82% relative humidity. All the cheeses were judged to be normal in both body and flavour.

Isolation of bacteria in pure culture. Cheese samples (10 g.), removed from uncontaminated cheese by aseptic techniques, were finely ground in sterile grinding tubes with sterile saline (10 ml.). Loopfuls were taken from this mixture and streaked on to plates containing a 2% lactose–yeast–phosphate agar (LYPA) prepared according to Hunter(2). They were then incubated for 3–5 days at 30°. Twenty colonies were picked off at random from each plate, inoculated into a skim-milk media enriched with yeast extract (Oxoid brand, 0·2%) and then examined microscopically after further incubation for 3–5 days at 30°.

Cultures. The original culture of A2 was isolated in September 1951, and subcultures from this were subsequently freeze-dried and kept in the collection of dairy bacteria at the Dairy Research Institute (New Zealand), as organism 'A2'. Subcultures have been

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deposited at the National Institute for Research in Dairying, Shinfield, near Reading, England, and catalogued as no. 559 of the National Collection of Dairy Organisms.

Bacteriological methods. During experiments, A2 was maintained in a yeast (0·2%) skim-milk media and was transferred into fresh media twice a week. The behaviour of the cocci were studied in both a 2% lactose–yeast–phosphate broth (LYPB) and on the LYPA media of Hunter (2). The fermentation characteristics were observed in a medium which consisted of a double-digest of casein prepared according to Davis (3) with the addition of 0·2% yeast extract and also in the tomato-juice medium as described by Briggs (4).

Biochemical methods. The production of gas (CO_2) was investigated following the methods of Gibson & Abdel-Malek (5) and Hayward (6). Lactic acid was estimated using the volumetric method of Friedemann & Graeser (7) with the modifications described by Neish (8); acetic acid was also estimated volumetrically following the distillation technique described by Olmsted, Whitaker & Duden (9). In a separate experiment, the lactic acid was isolated as the zinc salt by the method of Pederson, Peterson & Fred (10) and the rotation was determined. Diacetyl was measured using the method developed by Eggleton, Elsden & Gough (11). Catalase activity was tested for using the media and methods described by Felton, Evans & Niven (12) and Gutekunst, Delwiche & Seeley (13) for the pediococci and by Dacre & Sharpe (14) for some lactobacilli.

DESCRIPTION AND CHARACTERISTICS OF THE ORGANISM

Morphological characters

Shape, size and arrangement of the cells. After growth in the LYPB medium for 24 hr. at 30°, the majority of the cells were spherical in form and grouped in pairs (diplococci); single cells, tetrads and very occasionally a few short chains of not more than four to six cells were observed. The cocci varied in size between 1·2 and 1·8 μ in diameter and were non-motile.

Staining. The cells were Gram-positive; neither endospores nor flagella were detected and attempts to detect capsule formation by the use of several staining procedures were unsuccessful.

Cultural characters

Growth on solid media. On LYPA medium slopes at 30° after 5 days' incubation, a moderately strong, beaded to nodose growth occurred; the colonies were smooth, whitish, opaque and mostly about 0·8–1·0 mm. in diameter. After 7 days on LYPA medium plates at 30°, the colonies were from 1·0 to 1·5 mm. in diameter, circular, smooth and entire. On a nutrient agar slope, a slight nodose growth only took place at 30°.

Growth in stab. In LYPA medium at 30° after 5 days' incubation, growth was moderately strong, beaded to papillate and did not occur to any extent at the surface.

Growth in liquid media. In 2–3 days at 30° the organism developed well in LYPB media to produce a moderate turbidity; on continued incubation, the culture liquid subsequently partly cleared and a greyish-white deposit of cells collected.

Physiological characters

Relation to temperature. In yeast skim-milk and LYPB media, the optimum temperature for growth was 28–30°; the minimum temperature was 10° and the maximum temperature for growth falls between 40 and 45°.

Resistance to heat. The organism was killed by heating for 30 min. at 60° when grown in the yeast skim-milk medium for 3 days, but survived a similar treatment when grown in the LYPB medium.

Relation to oxygen. The organism was a facultative anaerobe.

Relation to pH *value.* In broth media and in Cheddar cheese, the optimum pH range for growth was from 5·0 to 6·0; no growth occurred at pH 4·0 or above pH 8·5.

Relation to salt concentration. The cocci grew in the presence of 4·0% sodium chloride but not in 6·5% or higher concentrations.

Biochemical characters

The methyl-red test was positive. In MacConkey broth the organism produced acid after 2 days but no gas was evolved in 7 days at 30°; nor was gas produced when tested by the methods of Gibson & Abdel-Malek (5) and Hayward (6). The Voges–Proskauer reaction for acetyl–methyl–carbinol (acetoin) formation was positive; diacetyl production was also shown to take place, e.g. in LYPB media, 15–20 p.p.m. were detected after 3–4 days' incubation at 30°. The indole test was negative, gelatine was not liquefied and nitrate was not reduced to nitrite by the organism. Catalase and amino-acid decarboxylases were absent.

Litmus milk reaction. A clot was formed in 18–20 days with some reduction of the litmus in 11–12 days at 30°. The organism clots 0·2% yeast skim-milk media in 5–6 days at 22°, in 3–4 days at 30° and in 3 days when incubated at 37°.

Growth in the presence of Teepol. The cocci grew well in the tomato-extract medium with the addition of 0·05% Teepol, but not in the presence of 0·1% Teepol (cf. Jameson & Emberley (15)).

Growth on sheep's blood agar. The organism showed good growth with β-haemolysis, but no green colour appeared in the medium surrounding the colonies.

Utilization of carbohydrates. In a double-digest of casein supplemented with a yeast or tomato extract, growth occurred and acid was formed at 30° from: arabinose, glucose, fructose, galactose, mannose, lactose, maltose and dextrin; glycerol was fermented slowly. No growth took place with raffinose, sucrose, trehalose, melibiose, xylose, sorbitol and mannitol while mannitol was not formed from fructose. The culture failed to hydrolyse starch, inulin and sodium hippurate; it hydrolysed aesculin and salicin, and arginine with the production of ammonia. Acetate, succinate, lactate and citrate were not utilized as a sole carbon source; the organism utilized citrate in the skim-milk and broth media only in the presence of a fermentable sugar.

The acids produced from glucose. The final pH in a 1·0% glucose-broth medium was 4·0–4·2. Both inactive lactic acid and acetic acid were produced from glucose in the molecular ratio 100:12. Gas-liquid partition chromatographic analysis (after James & Martin (16)) of the culture filtrate, after 3 and 7 days' incubation, indicated that acetic acid was the only volatile acid formed by the organism.

DISCUSSION

The genus *Pediococcus* according to Pederson (17) (see also Bergey (18)) should include all the Gram-positive, non-motile, non-spore-forming cocci which tend to form packets of four but which may occur as single or paired cells. These cocci are micro-aerophilic,

high-acid homofermentative lactic-acid producers and they do not reduce nitrates, liquefy gelatin or produce catalase. In a further study of some thirty-four strains of pediococci, Jensen & Seeley (19) found that these organisms all fermented trehalose and hydrolysed arginine and aesculin; none of them fermented glycerol or sorbitol, nor hydrolysed sodium hippurate or produced mucoid colonies on a sucrose–gelatin agar media. On a 2% glucose medium with the addition of tryptone, yeast extract and tomato juice, the final pH obtained for twenty of the cultures was between 3·7 and 4·0. The organism A 2 described in this paper has all these characteristics with the exception that trehalose was not fermented.

Further confirmation that A 2 belonged in the *Pediococcus* genus was obtained when following the key of Skerman (20) for the determination of the generic position of organisms. The additional distinguishing properties given in the key are that these Gram-positive cocci should produce at least 0·5–0·7% of acid when grown in a glucose–yeast–tryptone–phosphate broth by lowering the pH to about 4·0 and that the lactic acid produced is optically inactive. In particular, this latter property distinguishes A 2 from the *Leuconostoc* species. Additional reactions that differentiate A 2 from the leuconostocs are the non-fermentation of sucrose, and hence no slime formation and the non-production of gas when grown in a glucose–yeast–tryptone–phosphate broth (cf. Bergey (18); Skerman (20)).

Felton *et al.* (12) described the production of catalase by six authentic strains of pediococci when grown on a yeast-enriched medium containing only 0·05% of glucose. In a recent study by Niven (personal communication) of the pediococci occurring in fermented sausages, both catalase-positive and catalase-negative strains were found to be present. In spite of carrying out extensive tests for catalase production on many different types of media with varying pH (cf. Gutekunst *et al.* (13)), the organism A 2 and numerous other isolates always gave a negative reaction.

A comparison of the physiological and biochemical characteristics of A 2 with four authentic strains of *Pediococcus cerevisiae* Balcke (National Collection of Dairy Organisms, nos. 514 (ATCC 8081), 814 (ATCC 10791), 990 and 991) confirmed that in all probability it is a variant of this type species. The coccus described here differs from these four strains in not only being trehalose-negative, but also being less tolerant to low pH levels (4·2) and to high concentrations of Teepol (0·1%).

The pediococci comprise a group of micro-organisms that are of economic importance in the brewing industry (see Pederson (17); Tittsler *et al.* (21)) and they are encountered in a variety of fermenting vegetables, such as beans and cucumbers (Pederson (17)). In recent years they have been reported as occurring in the cecal faeces of turkeys (Harrison & Hansen (22)), the rumen of cows (Bauman & Foster (23)) and in summer sausages (Deibel & Niven (24)). The occurrence of strains of pediococci in Cheddar cheese and the fact that they are able to enhance the typical flavour (Dacre (25))* should stimulate further research on their role in cheese ripening and on the metabolism of this little-investigated but undoubtedly important genus.

SUMMARY

1. Cultural and biochemical details are given of a homofermentative species of lactic acid-producing bacteria, repeatedly isolated from typical maturing New Zealand Cheddar cheese.

* In this paper the organism A 2 is mistakenly referred to as a '*Leuconostoc* strain'.

2. The Gram-positive cocci, occurring mainly in pairs and tetrads in acid media, are greatly stimulated in growth by the presence of yeast or tomato extracts. The organism converts glucose into inactive lactic acid and smaller amounts of acetic acid.

3. With the exceptions that the organism does not ferment trehalose and is less tolerant to low pH and high concentrations of Teepol, all its characteristics are similar to those for the genus *Pediococcus*, in particular to the type species *P. cerevisiae* Balcke.

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730. A NOTE ON THE PEDIOCOCCI IN NEW ZEALAND CHEDDAR CHEESE

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(With 1 Figure)

The changes in the bacterial flora which take place in ripening Cheddar cheese, and in particular their relationships to the development of flavour, have been the object of many investigations (1, 2, 3, 4, 5, 6, 7). In connexion with studies concerned with the mechanism of flavour development in New Zealand Cheddar cheese (8, 9, 10, 11, 12), the progressive changes in the bacterial flora of ripening cheese were followed. Some preliminary observations, which might form the basis for further research, are presented in this note.

EXPERIMENTAL

Cheeses. Nine of the ten cheeses used were 10 lb. loaves manufactured from good-quality flash-pasteurized milk in the Institute's experimental cheese factory, between September 1951 and November 1953. Both single and mixed variant strains of *Streptococcus cremoris* were used as starters (Table 2). On removal from the press the day following manufacture, these cheeses were immediately cut up into pieces of approximately 350 g. each. The pieces were waxed and kept in glass jars with loose-fitting lids in the laboratory at room temperature. Cheese no. 10 (for manufacturing details see (10)) was an 80 lb. export type cheese and was kept in a cheese-curing room at the experimental factory. All the cheese samples were examined frequently during the ripening period and were judged to be normal in both body and flavour.

Bacteriological methods. Cheese samples (10 g.), removed from the interior of the pieces of cheese by aseptic techniques, were homogenized in sterile saline (10 ml.). Standard loopfuls were taken from this mixture and streaked onto duplicate agar plates (prepared according to Hunter (13)) and then incubated for 3 and 7 days at 30° C. Samples were plated three times a week during the first 4 months of ripening; in the case of cheese nos. 1 and 9, two further plates were streaked and incubated for 3 and 7 days at 22° C. After 4 months, one plate only was streaked and incubated for 7 days at 30° C. from samples taken once a week. Twenty colonies were picked off at random from each plate after incubation, inoculated into tubes containing a yeast (0·2 %, Oxoid brand) skim-milk medium and again incubated at 30° C. All these cultures were then broadly classified into the groups comprising the streptococci, lactobacilli and pediococci following essentially the schemes of Sherwood (8) and Pederson (14) (see also Skerman (15)).

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RESULTS AND DISCUSSION

(1) *The occurrence of pediococci in Cheddar cheese.* In addition to streptococci and lactobacilli, and occasionally leuconostocs and micrococci, organisms classified in the *Pediococcus* genus (Dacre(12)) were isolated and characterized from all the cheeses examined. An analysis of the distribution of the pediococci and the lactobacilli in ripening

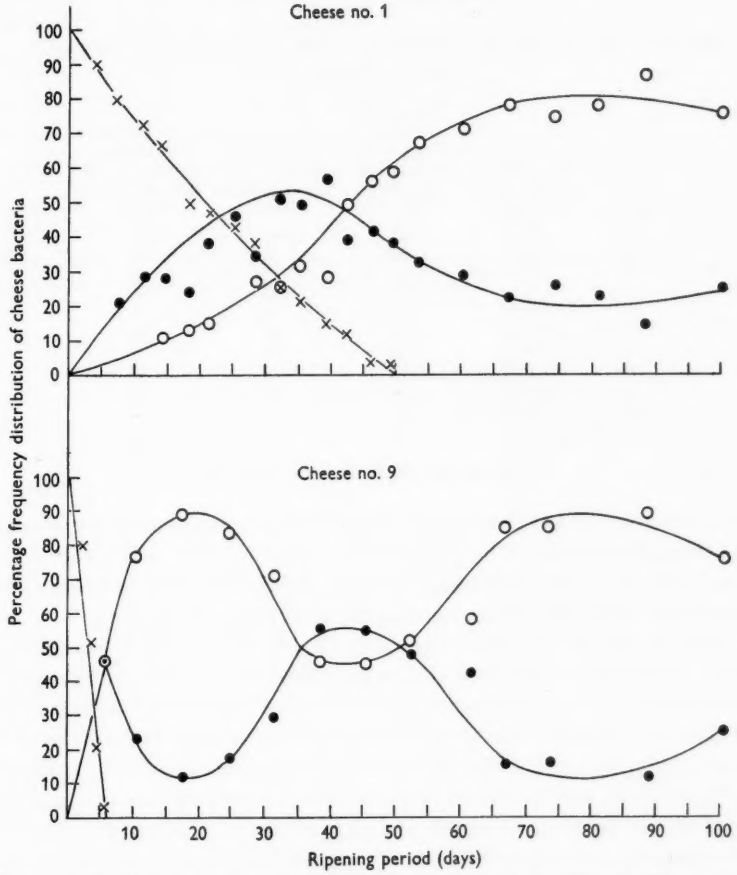

Fig. 1. Frequency distribution curves of the lactic-acid bacteria in ripening Cheddar cheese. ×——×, Streptococci; ○——○, lactobacilli; ●——●, pediococci. (Note. Not all the points are shown on the curves.)

cheese is set out in Table 1. It will be seen that the cocci gradually decrease in number but at about 6 months they constituted about a quarter of the total bacterial population of cheese. From the detailed analysis made of cheese nos. 1 and 9, frequency distribution curves were drawn (Fig. 1). The variable nature of the distribution of these two groups during the early days of ripening is clearly shown although not apparent from the figures of Table 1. In these two cheeses, however, the appearance and development of the flavour followed more closely the development of the pediococci rather than that of the

Table 1. *Percentage distribution of the lactobacilli and pediococci in ripening Cheddar cheese*

(Average of five cheeses.)

Age of cheese (months)	1*	2	3	4	5	6	9
% lactobacilli	61	69	73	76	80	76	75
% pediococci	33	31	27	24	20	24	25

* Streptococci 6% (cheese no. 1).

Table 2. *The appearance and disappearance of the lactic-acid bacteria in ripening Cheddar cheese*

No. of cheese	*Str. cremoris* strain used as starter	Time taken for the streptococci to disappear completely (days)	Time taken for the first appearance of the lactobacilli (days)	Time taken for the first appearance of the pediococci (days)
1	E8	50	14	5
2	HP	26	18	18
3	HP	12	10	8
4	K	10	6	5
5	HP/K	12	8	8
6	HP/K	16	14	20
7	HP/K	10	10	9
8	HP/K	16	7	8
9	KH/K	6	5	5
10	HP/K*	15	7	7
	Average time	17	10	9

* With the addition of *Str. faecalis* (see (10)).

lactobacilli. Moreover, it has already been shown that a *Pediococcus* culture (strain no. A2 which was initially thought to be a *Leuconostoc* (see Dacre (10)), when added to cheese milk along with the starter streptococci (strains HP and K of *Str. cremoris*), enhanced the development of Cheddar flavour during the early stages of ripening.

(2) *Changes in the bacterial flora of ripening Cheddar cheese.* The appearance and disappearance of the lactic-acid cheese bacteria was followed in ten different ripening cheeses. The time taken for the starter streptococci to disappear completely was found to be very variable (Table 2). Figures were obtained ranging from as early as 6 days up to 50 days (Fig. 1) and giving an average of 17 days for these organisms to die out. In all cases, the lactobacilli appeared within 18 days from the time of manufacture of the cheeses and always before the starter disappeared, except in cheese no. 7 where the times were the same (cf. Davis (4)). The pediococci generally appeared about the same time as the lactobacilli except in two of the cheeses examined. In cheese no. 1 they appeared first on the fifth day of ripening; in cheese no. 6 they did not appear until a further 6 days after the first appearance of the lactobacilli. It should be noted here that nine of the cheeses studied in this work were ripened at room temperature and not under normal cheese-ripening conditions.

SUMMARY

Organisms identified as *Pediococcus* strains were found to constitute about a quarter of the normal lactic-acid flora of ripening New Zealand Cheddar cheese. The development and the frequency distribution of these pediococci, in relation to the starter streptococci and lactobacilli were briefly studied.

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731. LACTOBACILLI IN CHEESE STARTER CULTURES

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For a survey of the numbers of lactobacilli present in milk used for cheese-making in creameries, counts were made on an acetate-agar medium (1) selective for lactobacilli. It was observed that the numbers of lactobacilli in the milk in the cheese vat sometimes greatly increased after the addition of the starter to the milk. When these starters were plated on acetate agar growth of lactobacilli, sometimes in large numbers, suggested contamination of the starters with these organisms. The starters in use at sixteen different creameries were then examined on acetate agar, and seven were found to contain lactobacilli. Representative colonies of these organisms were picked from each starter and these strains identified physiologically using the methods of Briggs (2) and Wheater (3, 4), and serologically using the group sera of Sharpe (5) and Sharpe & Wheater (6). Table 1 shows the numbers and species of lactobacilli present in the starters. Each starter was sampled several times, usually at monthly intervals.

The *mother* culture at creamery A was sampled. At creameries B and C the *mother* and the *bulk* cultures were examined and both were found to be contaminated. At creameries D, E, F and G the bulk starters only were examined.

In creamery A, where the mother starter was kept in an uncovered bottle or covered can before use, different species of lactobacilli were isolated at each sampling. *Lactobacillus fermenti* and *L. bulgaricus*, which do not grow at 15° C., would not have grown in the cheese (the usual ripening temperature is about 15° C.). The unclassified strain which was recovered at the next sampling was a low-temperature organism, which might have grown in the cheese.

From the starter at creamery B, *L. plantarum* and *L. brevis* were both isolated in small numbers at the first two samplings, but later *L. bulgaricus* appeared in large numbers.

At creamery C, *L. bulgaricus* was again isolated. A fresh mother culture of starter was brought into use after two samplings. At the next sampling this starter also contained large numbers of lactobacilli, showing that recontamination had occurred.

In creameries D and E, *L. casei* was found in the starters, but the numbers were small and contamination sporadic. The starter at creamery F contained *L. casei*, *L. plantarum* and *L. brevis*. These three species flourish in cheese, and are indeed the species most frequently isolated from it during ripening.

Although the numbers of lactobacilli in the starters were often small there was further opportunity for them to increase whilst in the vat, and samples taken from the milk in the vat showed that this was indeed happening.

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Six starters being used for cheese-making at the N.I.R.D. were examined, and found to contain no lactobacilli, and sixty-five commercial starters (as supplied by distributors of starters, some of them being freeze-dried) from different parts of the country and from the U.S.A. were also found to contain none of these organisms. Although all were vigorously growing starters, none was able to grow on acetate agar. This confirms the

Table 1. *Numbers and species of lactobacilli isolated from starters at different creameries*

Creamery	Date of sampling	Nos. of lactobacilli/ml. of starter milk	Species of lactobacilli isolated
A	7. xi. 57	>100,000	*L. fermanti*
	11. xii. 57	>1,000,000	Unclassified *Streptobacterium**
	19. ii. 58	>1,000,000	*L. bulgaricus*
B	13. xi. 57	12	*L. plantarum, L. casei*
	18. xii. 57	5	*L. plantarum, L. casei*
	15. i. 58	516,000	*L. bulgaricus, L. plantarum*
	11. ii. 58	>50,000	*L. bulgaricus*
	11. iii. 58	>500,000	Not examined
C	20. xi. 57	1,088,000	*L. bulgaricus*
	8. i. 58	5,000	*L. bulgaricus*
	27. ii. 58†	0	.
	26. iii. 58	672,000	Not examined
D	26. xi. 57	51	*L. casei*
	2. i. 58	2	Not examined
	12. ii. 58	0	.
E	11. xii. 57	20	*L. casei*
	29. i. 58	0	.
	20. ii. 58	100	Not examined
F	10. xii. 57	180	*L. casei, L. plantarum, L. brevis*
	29. i. 58	15	Not examined
	19. ii. 58	3	Not examined
G	4. ii. 58	>100,000	Not examined
	28. ii. 58	1,600,000	*L. bulgaricus, L. plantarum*
	6. iii. 58	142,000	*L. helveticus*

* Homofermentative low-temperature organism, neither *L. casei*, nor *L. plantarum*.
† New mother starter culture.

highly selective action of this medium. The absence of lactobacilli from these commercial starters at the time of purchase points to contamination in the creamery. Most of the species of lactobacilli isolated from the starters are commonly found in milk, which may be contaminated from many sources, such as dairy utensils, cheese, silage or saliva.

The frequent occurrence of *L. bulgaricus* as the contaminating lactobacillus in many of these starters is interesting. This species was isolated from four different starters, at two Welsh creameries, one English and one Scottish creamery, respectively. However, as it does not grow at the ripening temperature of Cheddar cheese its presence in starter is likely to be of no significance. It is commonly used together with *Streptococcus thermophilus* for the preparation of yoghurt. The streptococcus grows first and the resulting acid production and formation of suitable metabolites assists the later growth of the lactobacillus. A similar situation may obtain with starters in which the growth of *Str. lactis* and *Str. cremoris* may create conditions very favourable for the growth of *Lactobacillus bulgaricus*. These strains differed from Wheater's (3) description of this species in that they usually fermented trehalose and grew in 2% NaCl. None of them grew at 45° C.

It has been shown (7,8) that the inoculation of cheese-milk with certain strains of lactobacilli—e.g. strains of *L. casei*, *L. brevis*, *L. plantarum*—may affect the flavour of the cheese during ripening. Whilst some strains appear to improve the flavour, others may cause off-flavours, bitterness or weak body. The chance addition of contaminant lactobacillus strains adds one more hazard to the somewhat empirical methods of cheese-making, and is therefore to be avoided.

The ease with which lactobacilli appear to gain entrance to starter cultures in the creamery suggests laxity in aseptic techniques which might have more serious consequences. If contamination of starters with lactobacilli can so frequently occur it is likely that bacteriophage will also infect them.

We gratefully acknowledge the co-operation of those creamery proprietors who allowed us access to their creameries. Mr B. Reiter was kind enough to supply the sixty-five commercial starters which were examined.

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732. LACTOBACILLI IN CHEDDAR CHEESE

II. DUPLICATE CHEESES

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(With 8 Figures)

Previous work(1) on the lactobacillus flora of a Cheddar cheese showed that many serological types could occur in the cheese during ripening. Whilst some of these were distributed throughout the cheese, others occurred irregularly in pockets. Duplicate cheeses made from the same milk and under the same conditions have now been examined to find whether they contained similar patterns and distributions of serological types of lactobacilli. The lactobacillus flora of two pairs of duplicate cheeses is described here. Another pair of cheeses was also included, made under the same conditions, but with a known strain of *Lactobacillus* added in small numbers to the cheese-milk in one vat. The distribution of this organism was traced during cheese-making and the ripening of the cheese.

METHODS

Cheese-making

Three pairs of cheeses were made on different occasions from raw milk in twin vats with identical performances in all respects. The first two pairs (A and B; C and D) were each made from common bulk milks and under the same conditions. The third pair (J and H) was also made from a common bulk of milk under the same conditions, but cheese J was inoculated with a known strain of *Lactobacillus*. The organism used was serological type strain A41, an unclassified homofermentative strain previously isolated from cheese L2(1). Ten ml. of a 1/10 dilution of a 48 hr. culture of this organism, grown in yeast dextrose litmus milk, was added to the cheese-milk before renneting to give a concentration of 390 organisms/ml. of milk. A lid was kept on the vat whilst the inoculum was added, to prevent cross-contamination. During the making of these two cheeses samples were taken of the bulk milk, the milk in each vat just before renneting, the curd and whey at run-off and the curd at milling. These were plated on the selective acetate-agar medium.

For each of these experiments uniform conditions were maintained in the two vats during cheese-making.

Sampling of the cheeses during ripening

To reduce the effect of the irregular distribution in pockets of some lactobacilli, pooled samples of cheese, each consisting of three cores, were examined at each sampling. For cheeses A and B one pooled sample was taken from the top of each cheese at each sampling, but for cheeses C and D and cheeses H and J one pooled sample was taken from the top and also one from the bottom of each cheese at each sampling. The top was arbitrarily chosen as the face uppermost when the cheese was removed from the press,

but frequent turning during ripening ensured that there was no other difference between the two surfaces.

The top ½ in. of each of the three cores was removed, the remainder minced together in a sterile mincer, and 9 g. of this was homogenized (1).

Isolation of cultures

For the duplicate cheeses (A and B; C and D), counts were made on tomato dextrose agar (TDA) to obtain the 'total' counts of lactic-acid bacteria, and on acetate agar (AcA) for counts of lactobacilli. For cheeses H and J counts were on AcA only. Twenty colonies were picked from the AcA platings of each sample, and purified. The cultures were divided into broad groups by testing for growth at 15° and 45° and for the production of gas from glucose. The organisms were typed serologically (1) and fresh sera prepared against strains not falling into the previously defined types. As further type sera were produced it became necessary to test extracts against an increasingly large number of type sera; with the more potent sera it was possible to do screening tests with polyvalent sera, consisting of mixtures of three different types. The type strains were fully identified biochemically (1) and representatives of each type tested for a number of characteristic reactions.

RESULTS

In all experiments the conditions in the vats were very similar throughout the cheese-making process. The members of each pair of cheeses were consistently similar in flavour, body, texture and in pH throughout the ripening period.

Cheeses A and B

The counts on TDA and AcA are shown in Fig. 1. On each medium the counts for each cheese were similar in the earlier stages but differed appreciably at 90 and 179 days.

Lactobacilli. A total of 110 lactobacilli was isolated, 104 being divided into 13 serological types by precipitin tests. These types were identified biochemically (Table 1): five types were *Lactobacillus brevis*, three were *L. plantarum*, three were *L. casei* and two were unclassified low-temperature homofermentative lactobacilli. All these type strains reacted specifically with the appropriate *Lactobacillus* group sera of Sharpe & Wheater (2), except one *L. casei* type and the unclassified types, which did not react with any of the group sera.

Of the thirteen serological types, eleven were new types, but two were the same as had been found in cheese L2 (1). These were O 55 (*L. plantarum*) and T 21 (*L. brevis*). Strains of each of these types had biochemical reactions identical with those of the type strains isolated from cheese L2 and they fully absorbed the type sera.

In addition to the lactobacilli large numbers of Gram-positive cocci were isolated from these cheeses throughout ripening. As these cocci were a major part of the flora, most of them were retained for further examination. In the first sample at 15 days three of the cocci were shown to be *Leuconostoc* spp. and one was identified as *Pediococcus* sp. All further cocci isolated from these cheeses during ripening were also identified as *Pediococcus* spp. They were Gram-positive, catalase negative cocci in clusters and tetrads; all grew at 15° but not at 45°, and none produced gas from glucose. The *Leuconostoc* spp.

were Gram-positive, catalase negative cocci or coccobacilli, growing at 15° but not at 45°, and producing gas from glucose but not NH_3 from arginine.

The distribution of the different types of lactobacilli and lactic cocci in cheeses A and B (Fig. 2) showed that except for the regular occurrence of pediococci the only type which occurred in both cheeses was T21.

The types found were not only different in the two cheeses at a single sampling, but often varied in the same cheese at each time of sampling.

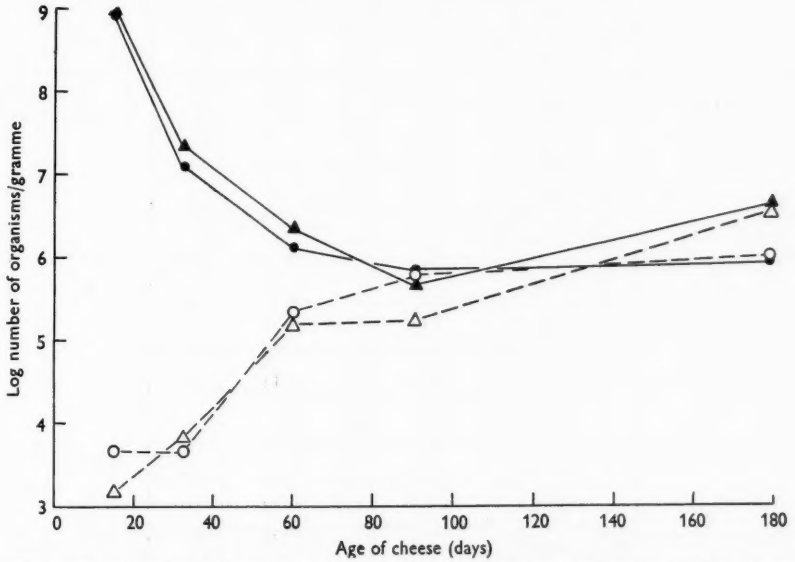

Fig. 1. The counts on tomato dextrose agar and acetate agar of samples from cheeses A and B taken throughout the ripening period. ●——●, Cheese A total counts on tomato dextrose agar; ○ - - - ○, cheese A counts on acetate agar; ▲——▲, cheese B total counts on tomato dextrose agar; △ - - - △, cheese B counts on acetate agar.

Cheeses C and D

The counts on TDA and AcA (Figs. 3 and 4) show that differences in numbers of more than tenfold occur in the different samples at any one time. This is particularly marked late in the ripening period. The differences between samples from the top and bottom of the same cheese were sometimes as great as those from different cheeses.

Lactobacilli. 459 lactobacilli were isolated, of which 447 fell into seven serological types (Table 1). These comprised three types of *L. brevis*, one of *L. plantarum*, one of *L. casei* and two of unclassified low-temperature lactobacilli. All except the unclassified strains reacted specifically with the appropriate group sera (2). One of the *L. brevis* strains was identical with type V7 which occurred in cheese L2 (1), and one of the unclassified strains was identical with type A101 found in cheese A.

The two main types of lactobacilli C106 (*L. plantarum*) and V7 (*L. brevis*) occurred frequently but somewhat irregularly (Fig. 5). In the middle of the ripening period the four samples had much the same flora, but in the early and later part some samples were quite different. For instance, at 7 days no lactobacilli were isolated from the bottom of

cheese D, type A101 which was not found again was isolated from the bottom of cheese C, type C106 occurred in the tops of both cheeses, and type V7 in the top of C only. At 16 days the main type in the bottom of cheese D was C106, whilst only V7 was isolated from the other samples.

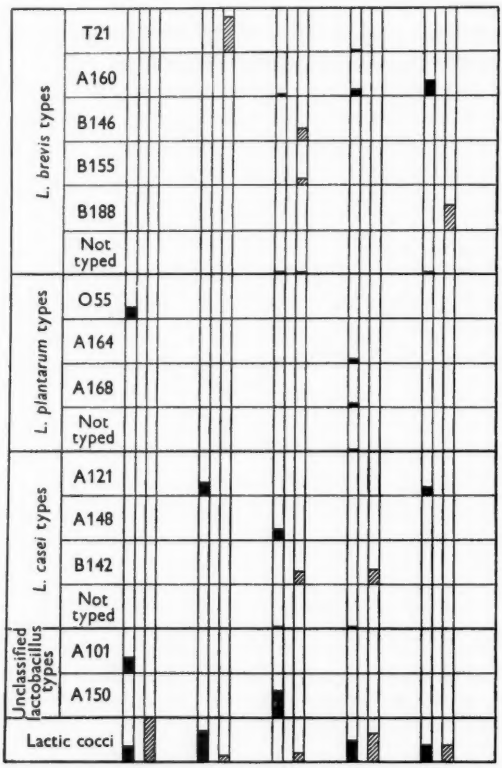

Fig. 2. The distribution throughout the ripening period of organisms isolated on acetate agar from cheeses A and B. ■, Represents five organisms from cheese A; ▨, represents five organisms from cheese B.

Only four organisms other than lactobacilli were isolated from AcA. They were Gram-positive, catalase negative homofermentative cocci, and were not further identified.

Cheeses H and J

Figs. 6 and 7 show the counts on AcA during the making and ripening of cheeses H and J. The addition of the lactobacillus to the cheese-milk J gave an increased count in the milk, but whilst the numbers in vat J were still higher in both curd and whey at run-off, similar numbers were found for both cheeses at milling. The number of organisms retained in the curd compared with the whey at run-off was 3·5:1 for H and 3·25:1 for J.

The counts of samples from both cheeses were much more similar than in the other pairs of cheeses throughout ripening.

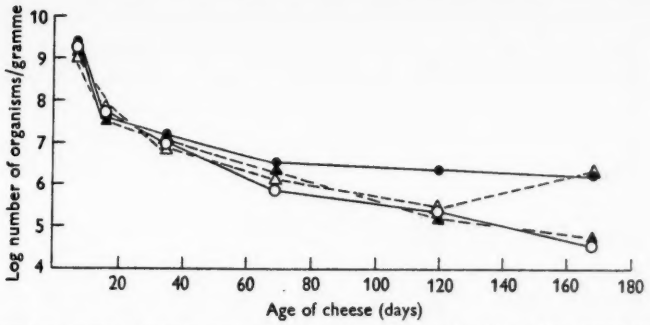

Fig. 3. The counts on tomato dextrose agar of samples from cheeses C and D taken throughout the ripening period. ●——●, Cheese C (top); ▲ - - - ▲, cheese D (top); ○——○, cheese C (bottom); △ - - - △, cheese D (bottom).

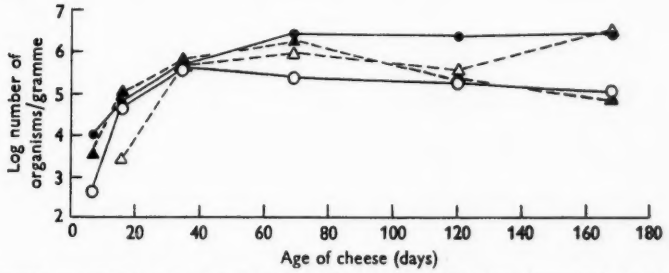

Fig. 4. The counts on acetate agar of samples from cheeses C and D taken throughout the ripening period. ●——●, Cheese C (top); ▲ - - - ▲, cheese D (top); ○——○, cheese C (bottom); △ - - - △, cheese D (bottom).

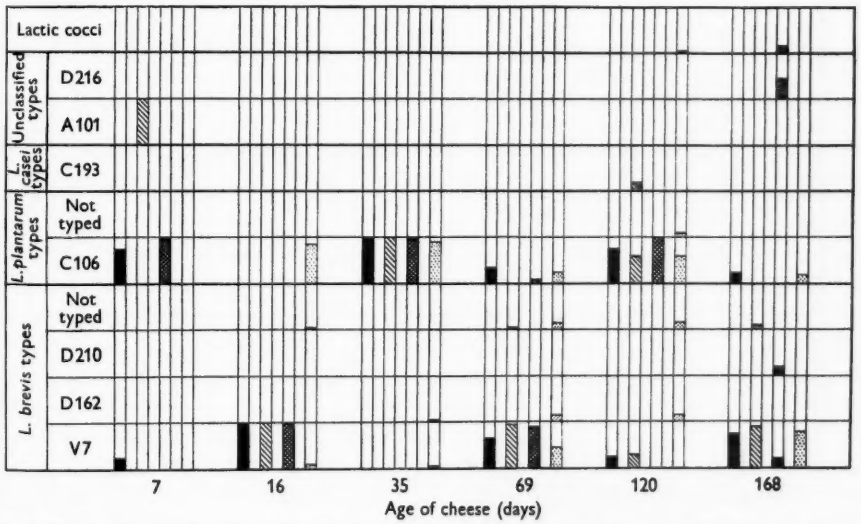

Fig. 5. The distribution throughout the ripening period of organisms isolated on acetate agar from cheeses C and D. Top of cheese C, ■; bottom of cheese C, ▧; top of cheese D, ▩; bottom of cheese D, ▤. These symbols represent five organisms.

Table 1. *Biochemical reactions of type strains and representatives of type strains isolated from the cheeses*

	Cheeses A and B													Cheeses C and D						Cheeses H and J	
Lactobacillus group ...	*L. brevis*					*L. plantarum*			*L. casei*			Unclassified		*L. brevis*			*L. plantarum*	*L. casei*	Unclassified	*L. plantarum*	Unclassified
Type strain ...	A160	B146	B155	B188	T21	A164	A168	O55	A121	A148	B142	A101	A150	V7	D162	D210	C106	C193	D216	H268	A41
No. of strains in selected tests ...	5	3	3	5	5	2	2	4	5	4	4	4	5	9	4	4	10	4	4	14	11
X Growth at 15° C.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
X Growth at 45° C.	−	−	−	−	−	−	−	+	±	±	±	−	−	−	−	−	−	−	−	−	−
X Gas from glucose	+	+	+	+	+	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−
X NH_3 from arginine	+	+	+	+	+	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−
X Growth in 0·1% Teepol	+	+	+	+	+	+	+	+	−	−	−	−	−	±	−	+	+	−	−	+	−
Growth in NaCl 4%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6%	−	−	−	+	−	+	+	+	+	+	+	±	+	+	−	−	+	+	+	+	+
8%	−	−	−	−	−	+	+	+	−	+	+	±	−	−	−	−	+	−	−	+	−
H Growth in YDLM	No change	No change	Slow ACR	Slow ACR	Slow AC	Slow ACR	Slow ACR	Med. ACR	Rapid ACR	Rapid ACR	Rapid ACR	Slow AC	Med. ACR	Slow A	No change	Slow ACR	Slow ACR	Rapid ACR	Slow ACR	Slow ACR	Slow ACR
X Hydrolysis of aesculin	−	−	−	−	±	+	+	+	+	−	+	−	−	±	±	±	+	+	−	+	−
Hydrolysis of hippurate	+	+	+	+	+	+	+	+	−	+	+	−	−	−	+	+	+	−	+	+	−
Production of acid from:																					
Amygdalin	−	−	−	−	−	+	+	+	+	+	+	±	−	−	−	−	+	+	+	+	+
X Arabinose	±	±	+	+	+	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−	−
X Cellobiose	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−
Dextrin	−	−	−	−	−	+	+	±	±	+	−	−	−	−	−	−	+	−	−	+	−
Dulcitol	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−
Glycerol	−	−	−	−	−	±	±	+	−	±	±	−	−	−	−	−	−	+	−	+	−
Inulin	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−
X Lactose	−	−	+	−	±	+	+	+	±	−	+	+	+	+	−	±	+	+	−	+	+
H Mannitol	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+
Mannose	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−
Melezitose	−	−	−	−	±	−	+	+	+	+	+	−	−	−	+	+	+	+	−	+	±
X Melibiose	−	−	+	+	±	+	+	+	−	−	−	−	−	−	+	+	+	−	−	+	−
H Raffinose	−	−	−	−	±	±	±	+	−	−	−	−	−	−	+	+	+	−	−	±	−
H Rhamnose	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+
H Salicin	−	−	−	−	−	+	+	+	+	+	+	±	−	−	−	−	+	+	−	+	−
Sorbitol	−	−	−	−	−	+	+	+	−	+	+	−	−	−	−	−	+	+	+	+	−
Sorbose	−	−	−	−	−	−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−
Sucrose	−	−	−	−	−	±	+	+	±	±	+	−	−	−	+	+	+	+	+	+	+
H Trehalose	−	−	−	−	−	+	+	+	+	+	+	−	+	−	−	−	+	+	+	+	−
B Xylose	+	+	+	+	+	−	−	+	−	−	−	−	−	+	+	+	−	−	−	−	−

Notes. ± =slight, late or variable reaction. In YDLM test: slow =more than 4 days to produce action; med. =3–4 days to produce action; rapid =2 days to produce action; A =acid; C =clot; R =reduction.

This table shows the reactions of the type strains. Representatives of the type strains were also examined in a selected number of tests which are shown as follows: X, representative strains of all groups tested; B, representative strains of *L. brevis* only tested; H, representative strains of homofermentative group only tested.

All strains tested fermented dextrose, galactose, laevulose and maltose.

Fig. 7. O- (bo

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Lactobacilli. 710 strains were isolated during cheese-making and from the cheeses, of which 195 were *Lactobacillus* A41. These latter strains were isolated only from J samples. Of the remaining strains, 488 belonged to the same serological type H268 (*L. plantarum*) (Table 1), two were *L. brevis* type V7, twelve were untyped *L. plantarum*, eight were untyped *L. casei*, three were low-temperature homofermentative lactobacilli, and two were untyped *L. brevis*.

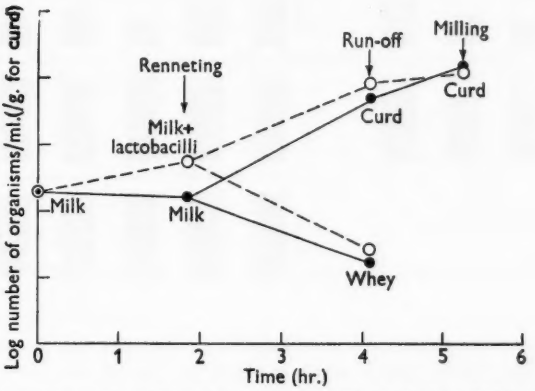

Fig. 6. The counts on acetate agar of samples from cheeses H and J taken during the cheese-making process. ●——●, Vat H; ○ - - - ○, vat J.

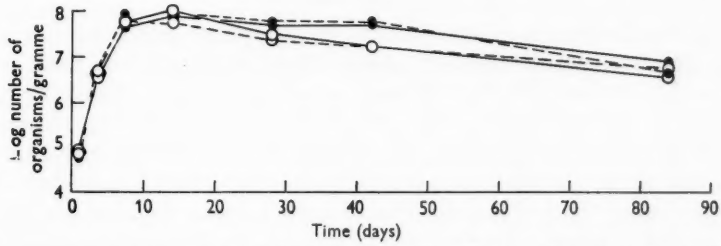

Fig. 7. The counts on acetate agar of samples from cheeses H and J taken throughout the ripening period. ○——○, Cheese H (top); ●——●, cheese J (top); ○ - - - ○, cheese H (bottom); ● - - - ●, cheese J (bottom).

Type H268 occurred most frequently; it was present in the milk and remained as the predominant flora in cheese H until, at 168 days, it was found to be partly replaced by other lactobacilli, including type V7, which also occurred in much larger numbers in cheese L2(1) and in cheeses C and D. With the inoculated cheese J, type A41 was isolated from all milk and cheese samples. H268 was also found in all these samples, usually in slightly smaller numbers than A41. The samples from the tops and bottoms of the cheese usually showed similar proportions of A41 and H268.

Thirty strains of lactic cocci were isolated. Twenty-one of these were leuconostocs, isolated mainly during cheese-making and also in the 2-day-old cheese. The other nine strains were pediococci and were isolated during cheese-making, but were not found in the cheese itself.

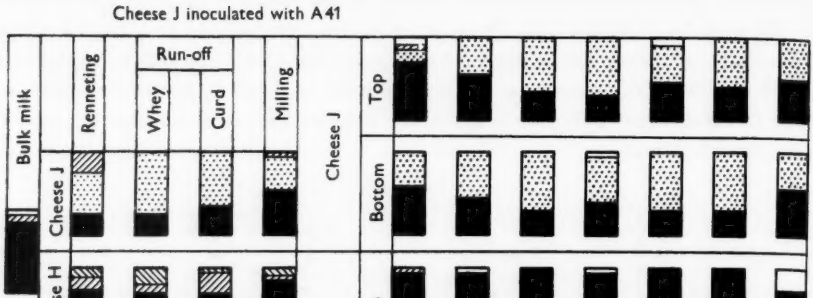

Fig. 8. The proportions of the different organisms isolated on acetate agar from samples of cheeses H and J during cheese-making and the ripening period. ■, H 268; ▤, A 41; ▨, *Leuconostoc* spp.; ▧, *Pediococcus* spp.; □, miscellaneous lactobacilli. [Approx. twenty colonies identified from each sample.]

DISCUSSION

In studying the distribution of lactobacilli in these duplicate cheeses serological typing was again found to be rewarding and reliable. Many new types were found, although three (types V 7, O 55 and T 21) were the same as had been found in cheese L 2 (1). One of these types, V 7, was isolated from cheeses C and D made 13 months after cheese L 2, and from cheese H made 19 months later. The other two types, O 55 and T 21, were isolated from cheeses A and B made 10 months later. Type A 101, originally isolated from cheese A, recurred in cheese D made 3 months later. This tendency for types to recur in cheeses suggests a resident lactobacillus flora gaining access to the cheese during making.

Of the new serological types, six were *L. brevis*, four were *L. plantarum*, four were *L. casei* and three were unclassified low-temperature homofermentative lactobacilli. *L. casei*, which was reported by Davis (3) to constitute a large part of Cheddar cheese flora, was not found in large numbers in these cheeses. *L. plantarum* was found in small numbers in cheese A, but in large numbers in cheeses C and D, and H and J. *L. brevis* occurred in cheeses A and B, and in C and D, but only infrequently in H and J.

The serological types of lactobacilli isolated from duplicate cheeses A and B, and C and D, varied and did not show similar patterns. From cheeses A and B a number of different serological types were isolated on different occasions, and only one type (T 21) was common to both cheeses. From cheeses C and D two main types were isolated, but they did not always occur at the same time in each sample, and samples taken from the tops and bottoms of the cheeses showed that there was as much variation within the cheeses as between them. The milks from which the duplicate cheeses A and B and also C and D were made had very low counts of lactobacilli, of the order of less than 1/ml. This suggests that in the curd the lactobacilli were distributed at random in the threads

of the casein gel (4), and that colonies formed around the sites of initial infection, thus giving rise to pockets which would vary in type within and between the cheeses. If the initial numbers of lactobacilli had been high the different types should have been well distributed throughout the curd and given more uniform counts and types in the cheese.

In cheeses H and J this was demonstrated in two ways. The milk used in the making of these cheeses had an initial lactobacillus count of almost 200/ml., which comprised mainly one type, H268. In the control cheese H this type was isolated in large numbers up to 6 months in all samples from both the top and the bottom. At 168 days the sample showed that some other types had developed which presumably had been greatly outnumbered in the original milk.

In cheese J an inoculum of a known serological type of *Lactobacillus*, A41, was added to simulate a natural infection. Once again the samples from the tops and bottoms of the cheese at each sampling were uniform in numbers and types. This suggests that the variability of the cheese flora would be reduced if the numbers in the milk were sufficient to give a good distribution throughout the curd. Variability in the distribution of lactobacilli in duplicate cheese could thus be eliminated by the addition of a small inoculum of a known *Lactobacillus* to the cheese milk.

A comparison of the numbers of lactobacilli occurring in these cheeses during ripening showed that a difference of almost a 100-fold could occur between samples taken at the same time from the top and bottom of the same cheese. Hiscox, Harrison & Wolf (5) have shown that the bacterial flora of a Stilton cheese is concentrated in irregularly scattered colonies throughout the cheese. Mabbitt (6) found that bacteria were irregularly scattered in Cheddar cheese, and our previous work (1) indicated that localized pockets of lactobacilli may occur. The size of our samples, and the pooling of three cores, should obviate some of this irregularity, but little significance should perhaps be attached to lactobacillus counts alone when unrelated to other data.

The differences in lactobacillus flora between the different pairs of cheese were not reflected in differences in flavour. This appears to indicate that if lactobacilli are involved it is not specific serological types or even species which give rise to differences in flavour. Rather this similarity in flavour suggests either that the production of flavour is a function of many different lactobacilli, or that it is determined by factors other than lactobacilli.

Large numbers of an organism physiologically identified as a pediococcus were isolated from cheeses A and B on AcA. Mabbitt & Zielinska (7) did not test the growth of pediococci on this medium, but did note that some *Leuconostoc* spp. would grow. These pediococci occurred in large numbers in both cheeses throughout the ripening period. They were also isolated from the cheese milk and curd of H and J. The growth of these pediococci on the acetate medium may indicate a metabolism similar to that of the lactobacilli and they may play a similar role in cheese. Leuconostocs were found in the cheese-milk and occasionally in the earlier stages of ripening of the cheese.

SUMMARY

The lactobacillus flora of two pairs of duplicate cheeses, each pair being made under the same conditions and from the same milk, was examined during ripening. A different distribution of serological types of lactobacilli was found not only within a pair of cheeses but in the top and bottom of the same cheese. A third pair of cheeses, to one of which

a known *Lactobacillus* had been added, was found to have a more regular distribution of lactobacilli owing to greater numbers being present in the cheese milk.

We wish to thank Dr A. T. R. Mattick for his interest and helpful advice in this work, Dr L. A. Mabbitt and Miss Helen R. Chapman for designing the twin vats, Miss Helen R. Chapman for making the cheese, and Miss E. J. Adams for technical assistance.

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733. LACTOBACILLI IN CHEDDAR CHEESE

III. THE SOURCE OF LACTOBACILLI IN CHEESE

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Lactobacilli occur in large numbers in cheese(1,2,3,4) and may be responsible for or influence the formation of flavour. It is therefore desirable to find the source of the lactobacilli and the route by which they enter the cheese, so that the cheese flora may be controlled.

It has been shown previously that lactobacilli occur in dairy utensils, cowyard dust and grasses(5), in silage(6,7,8), on fodder plants(9,10) and in milk(11,12). Bacteria, although not specifically lactobacilli, have been constantly demonstrated in the air of dairy plants(13) and saliva, a potential source of air contamination, is known to contain large numbers of lactobacilli(14,15). There are thus many sources from which lactobacilli in cheese might originate, but apart from Dolezalek(16), who showed that the same species of lactobacilli occurred on plants as in the cheese in particular districts, nobody has traced the actual source of these organisms.

In the course of our experiments previously reported(1,2) we sampled various sites to isolate lactobacilli which might be gaining access to the cheese, particularly during making. After identification these lactobacilli were tested serologically with the specific type sera(1,2) to see if they were identical with any of the serological types isolated from the cheeses.

METHODS

Isolation of lactobacilli

Milk. Preliminary experiments indicated that the numbers of lactobacilli isolated were likely to be small so several techniques were adopted to ensure their isolation. For direct counts 1 ml. amounts of milk were plated in acetate agar (AcA) in ten replicates. These were incubated for 5 days at 30° C. under 90% H_2+10% CO_2.

Enrichments were made by adding ten 1 ml. amounts of milk to tubes of acetate broth (AcB), incubating for 1 week at 30° C., and streaking on AcA.

To allow small numbers of lactobacilli which might be present to multiply sufficiently to be detected by plating, the milk itself was dispensed in 1, 5, 10 and 100 ml. amounts in screw-cap bottles and incubated at 30° C. for 1 month. Cultures were then isolated by streaking on AcA. It was thought that in this way the sequence of events in cheese ripening might be followed, with the natural streptococci multiplying first and the lactobacilli growing afterwards.

In some experiments a Yeastrel and glucose solution was added to the milk before incubation to encourage the growth of the lactobacilli.

Most of the milk samples were from a single herd at the National Institute for Research in Dairying, but two samples were from bulk tanker milks.

Saliva. The undiluted saliva and tenfold and 100-fold dilutions were plated in AcA and incubated as before at 30° C. This temperature was used to encourage the growth of lactobacilli likely to grow in cheese. After counting, two colonies of each different type, as judged by colony appearance, were picked into AcB.

Air. The air was sampled by means of a slit sampler similar to that described by Bourdillon, Lidwell & Thomas (17). Preliminary trials indicated that runs of at least ½–1 hr. were needed to give countable plates. When using AcA plates for this period it was found that the air flow caused sufficient desiccation for the acetate to crystallize which inhibited the growth of the lactobacilli. The method of Wolf, Nichols & Ineson (18) was tried in which gelatin acts as a collecting layer and is subsequently liquefied and plated. This was unsatisfactory because the air flow was strong enough to force the gelatin towards the sides of the plate, and desiccation was extreme. The method finally adopted consisted of using a layer of 2% agar as a collecting medium, and after exposure in the slit sampler, pouring 10 ml. of AcA over the exposed surface.

Cheese vats. Selected areas were swabbed with a moistened swab which was then enriched in AcB.

Hay. Individual fragments of hay were immersed in AcA which was just about to set.

Identification of lactobacilli isolated

Colonies from these different sources were picked into AcB and then purified by streaking on tomato dextrose agar (TDA). When the cultures were growing vigorously in tomato dextrose broth (TDB) they were classified using the tests in Table 1 and Table 2.*

Table 1. *Differentiation of lactic acid organisms isolated on acetate agar*

Group	Morphology	Catalase reaction	Growth at 15° C.	Growth at 45° C.	Gas from glucose	NH_3 from arginine	Serological groups
Homofermentative lactobacilli (high temperature)	Gram + bacilli	−	−	+	−	−	A, E
Homofermentative lactobacilli (low temperature)	Gram + bacilli or coccobacilli	−	+	−	−	−	B, C, D
Heterofermentative lactobacilli (*L. fermenti*)	Gram + bacilli	−	−	+	+	+	F
Heterofermentative lactobacilli (*L. brevis*)	Gram + bacilli or coccobacilli	−	+	−	+	+	E
Leuconostoc spp.	Gram + cocci or coccobacilli, often in chains	−	+	−	+	−	.
Lactic cocci (pediococci)	Gram + cocci in pairs, tetrads or clusters	− or + (weak)	+	−	−	+ or −	.
Streptococci	Gram + cocci in chains	−	+ or −	+ or −	−	+ or −	.

All the low-temperature homo- and heterofermentative lactobacilli were tested for serological type reactions using antisera prepared against the lactobacilli isolated from cheese (1,2). Any reactions which occurred were confirmed by absorption tests and by the biochemical tests in Table 3.

RESULTS

Lactobacilli isolated from different sources

Milk. Six milks from the same single herd, including the milks used to make cheese A, B, C, and D (2), were sampled between March and June 1956. These were found to have

* Any organism which grew better at 37° than at 30° C. was tested at the former temperature.

Table 2. *Differentiation of high-temperature homofermentative lactobacilli*

Test	Species				
	L. acidophilus	*L. bulgaricus*	*L. helveticus*	*L. lactis*	*L. salivarius*
Growth at 15° C.	−	−	−	−	−
Growth at 45° C.	+ (−)	+	+	+	+
Hydrolysis of aesculin	+	−	−	+ or −	+ or −
Fermentation of:					
Cellobiose	+	−	−	−	−
Maltose	+	−	+	+	+
Mannitol	−	−	−	−	+
Melezitose	−	−	−	−	−
Salicin	+	−	−	+	+ or −
Sucrose	+	−	−	+	+
Serological group	−	A	A	E	−

Table 3. *Differentiation of low-temperature homofermentative lactobacilli*

Test	Species		
	L. casei	*L. casei* var. *rhamnosus*	*L. plantarum*
Growth at 15° C.	+	+	+
Growth at 45° C.	− or +	+	−
Hydrolysis of aesculin	+	+	+
Growth in 0·1% Teepol	−	−	+
Fermentation of:			
Cellobiose	+	+	+
Mannitol	+	+	+
Melibiose	−	−	+
Raffinose	−	−	+ or −
Rhamnose	−	+	+ or −
Salicin	+	+	+
Sorbose	− or +	+	−
Trehalose	+	+	+
Formation of acid clot in YGLM*	Rapid	Rapid	Slow
Serological group	B, C	C	D

* YGLM = Yeastrel glucose litmus milk.

low counts on AcA varying from 0–10/ml. Most of the colonies picked from these plates failed to grow in AcB.

Lactobacilli were isolated from some of the enrichment broths and some of the incubated milk samples, indicating that the number of lactobacilli for most of the samples was of the order of one in 5–10 ml. In only one milk sample did the addition of Yeastrel and glucose to the milk before incubation enable lactobacilli to be isolated from smaller amounts of milk than in the corresponding untreated samples. Incubation of samples for 4 months instead of 1 month did not increase the numbers or species isolated.

Many of the organisms isolated were leuconostocs and some were clostridia. Only twenty-six lactobacilli were isolated from the six milk samples using all the methods cited. Of these thirteen were *Lactobacillus brevis*, six were *L. fermenti*, two were *L. casei*, two were unclassified low-temperature homofermentative lactobacilli and three were high-temperature lactobacilli. The two low-temperature homofermentative strains did not resemble either *L. casei* or *L. plantarum*. The three high-temperature homofermentative strains, when tested as in Table 2, gave the reactions of *L. salivarius*. When tested more

fully, using *L. salivarius* ATCC 11742 (one of Rogosa's original strains) as a comparison, they were found to differ by failing to ferment galactose, lactose, melibiose, raffinose, sorbitol and trehalose, and by failing to hydrolyse aesculin.

In later experiments in November–December 1956, four milks were tested with very different results. Two of these were bulk tanker milks, and two were from the same herd as the earlier milks. These gave counts on AcA of 8–360/ml. and enrichment methods were unnecessary.

Table 4. *The number and groups of lactic-acid bacteria isolated from different milks in November–December* 1956

Isolation method	Enrichment	Direct plating			
	I*	I*	II*	III†	IV†
Milk sample	15. xi. 56	15. xi. 56	22. xi. 56	28. xi. 56	5. xii. 56
Count/ml.	—	10·5	362	7·9	190
No. of strains isolated	10	104	20	72	20
Groups isolated (%):					
Streptobacteria	70	10	90	61	90
Thermobacteria	—	1	—	—	—
L. brevis	—	2	10	3	—
L. fermenti	10	4	—	—	—
Leuconostoc spp.	20	77	—	—	10
Pediococcus spp.	—	2	—	12	—
Streptococcus spp.	—	4	—	24	—

* Bulk tanker milk. † Single herd milk.

Table 5. Lactobacillus *species isolated from saliva*

Species	Number isolated	% of total isolated	% of samples in which occurred
L. acidophilus	41	38	60
L. fermenti	29	27	55
L. casei	26	24	60
L. lactis	7	6	15
L. brevis	3	3	10
L. salivarius	1	1	5
L. plantarum	1	1	5

Table 4 shows the counts and the species isolated from these later samples. A comparison of milk sampled on 15 November and examined by two different methods showed that of the organisms isolated by direct plating 77% were leuconostocs and 10% low-temperature homofermentative lactobacilli, while by the enrichment method 70% were low-temperature homofermentative lactobacilli and only 20% were leuconostocs. The proportions of the species isolated differed according to the method of examination, presumably because of the ability of the lactobacilli to outgrow the leuconostocs under the conditions of enrichment.

In this set of milk samples most of the lactobacilli isolated were low-temperature homofermentative species which were not further subdivided, while in the earlier sets of samples *L. brevis* was the commonest species.

Saliva. The counts on AcA varied from 30 to 60,000/ml., but were generally of the same order for one person sampled on different occasions. From twenty samples 108 lactobacilli were isolated, which were identified as shown in Table 5. In many cases organisms picked from the AcA plates failed to grow in AcB incubated at 30° or 37° C.

Air. Table 6 shows the different organisms isolated from the air of the dairy and cowshed. The counts were generally low in the N.I.R.D. dairy but were higher in the cowshed. In the latter the counts were related to the activity in progress. Higher counts were recorded when the cows were entering or leaving the shed, when feed was being distributed and when manure was being removed, whilst lower counts were found during milking itself and when there was no activity of any kind.

Some of the high counts recorded on 13 and 14 June were due to the growth of organisms which caused small splits in the agar but did not form visible colonies. It was found that these splits disappeared on standing, and in subsequent samples the colonies were not counted until the splits had gone. Organisms were isolated from several of these splits and were found to be *Clostridium butyricum*.

In one instance (22 November) a large increase in the number of lactobacilli in the air occurred at one period of sampling, when lactobacilli were being stirred into an inoculated vat. In a later experiment a lid was kept over the vat while the inoculum was stirred in, and no increase in air count occurred.

Cheese-making vats. No lactobacilli were isolated from the vats at the N.I.R.D.; these vats are always steamed before use.

Hay. Twenty-five organisms were isolated from hay, of which two were *L. brevis* and one was a pediococcus. Twenty-two were low temperature homofermentative lactobacilli, twenty-one of these reacting with the group D serum (*L. plantarum*) of Sharpe & Wheater (19) and one reacting with the group B serum (*L. casei*).

Serological typing of isolates from different sources

Milk. In cheeses H and J (2) most of the lactobacilli isolated were the same type (H 268) as that found in the milk in large numbers. When lactobacilli were in low numbers in the milk it was harder to trace the cheese lactobacilli to their source. None of the lactobacilli isolated from cheeses C and D were found in the milk used to make them. In the milk of cheese A and the whey from cheese B strains of *L. brevis* type T 21 were found and this was the only serological type of lactobacillus which was isolated from both of these cheeses. This type had previously been isolated (1) from a cheese made 10 months before.

Saliva. Although many lactobacilli capable of growth in cheese were isolated from saliva none of the serological types found in the cheeses could be traced to this source.

Air. Of the 152 isolates from air eight were serological types which were found in cheese. One was identical with A 148 (*L. casei*) and was isolated 3 months after the cheese in which it occurred was made. Four were identical with C 106 (*L. plantarum*) and were isolated from the dairy air on the day the cheeses in which it occurred were made. Another was identical with H 268 (*L. plantarum*) and was isolated on the day cheeses H and J were made. It is likely, however, that the milk which contained large numbers of this type had contaminated the air. One *L. brevis* (type V 7) was isolated from the air of the dairy a fortnight before cheeses C and D (2) were made and more than a year after cheese L 2 (1) was made, this type occurring in these three cheeses and also in cheese H (2). Another *L. brevis* (type T 21) was isolated from the air of the dairy 9 and 21 months after cheeses in which it occurred were made.

Hay. None of the isolates from hay were identical with any of the serological types found in cheese.

Table 6. *The numbers and groups of different organisms isolated from air by means of a slit sampler*

Date	Source	Count	Length of run (hr.)	No. of organisms isolated	Group of organisms isolated: Strepto-bacteria*	Thermo-bacteria†	*L. brevis*	*L. fermenti*	*Leuco nostoc* spp.	*Pedio-coccus* spp.	Other species isolated
8. iii. 56	N.I.R.D. dairy	2	0·5	2	2	.	.	.	.	.	.
11. vi. 56	N.I.R.D. dairy	8	1	6	2	.	1	.	3	.	.
		18	1	16	13	1	1	.	1	.	.
12. vi. 56	N.I.R.D. dairy	2	1	2	.	.	.	.	1	1	.
		4	1	3	3	.	.	.	.	.	.
		1	1	1	.	.	.	.	1	.	.
		2	1	2	2	.	.	.	.	.	.
		4	1	4	1	.	.	.	2	1	.
13. vi. 56	N.I.R.D. cowshed	24	1	12	6	.	4	.	1	1	.
		17	1	13	3	.	2	.	5	2	1 unidentified
		86	1	9	3	.	1	.	4	.	1 unidentified
		98	1	8	4	.	2	.	.	1	1 *Cl. butyricum*
14. vi. 56	N.I.R.D. cowshed	81	0·5	.	.	.	.	.	.	.	.
		>100	0·5	.	.	.	.	.	.	.	.
		10	1	.	.	.	.	.	.	.	.
15. vi. 56	N.I.R.D. dairy	11	1	4	2	.	1	.	1	.	.
		6	1	.	.	.	.	.	.	.	.
27. vi. 56	N.I.R.D. cowshed	3	0·5	1	.	.	.	.	1	.	.
		8	0·25	3	2	.	.	.	.	.	1 *Cl. butyricum*
		5	0·25	1	.	.	.	.	.	1	.
		1	0·25	1	.	.	.	.	.	.	1 *Cl. butyricum*
		3	0·25	2	.	.	.	.	2	.	.
28. vi. 56	N.I.R.D. dairy	2	1	1	.	.	.	.	.	.	1 *Cl. butyricum*
		5	1	5	2	.	1	.	2	.	.
		4	1	2	1	.	.	.	1	.	.
		3	1	3	1	.	.	.	1	.	.
		1	1	1	1	.	.	.	.	.	.
		11	1	9	4	2	.	1	1	.	1 *Cl. butyricum*
19. xi. 56	University dairy	0	0·25	.	.	.	.	.	.	.	.
		16	0·33	13	1	.	.	1	10	.	1 unidentified
		8	0·5	8	.	.	.	.	7	.	1 unidentified
22. xi. 56	University dairy	25	1	.	.	.	.	.	.	.	.
		235	1	.	.	.	.	.	.	.	.
		3	1	.	.	.	.	.	.	.	.
		13	1	.	.	.	.	.	.	.	.
		67	1	.	.	.	.	.	.	.	.
5. xii. 56	N.I.R.D. dairy	4	1	4	1	1	1	.	.	.	1 *Streptococcus*
		2	1	2	.	.	1	.	.	.	1 *Micrococcus*
		2	0·5	2	1	.	1	.	.	.	.
		1	1	1	.	.	.	.	.	.	1 *Streptococcus*
		4	1	4	1	.	1	.	1	.	1 *Streptococcus*
		2	1	1	.	.	.	1	.	.	.
		9	1	6	1	1	.	2	.	.	1 *Streptococcus* 1 yeast
				152	58	5	17	5	45	7	15

DISCUSSION

Although previously it was possible to isolate lactobacilli from a variety of sites, it could not be determined if these were actually the strains occurring in cheese. The use of a selective medium (AcA) for isolation, combined with serological typing, is a means of tracing accurately the source of these organisms.

In these studies we have traced serological types of lactobacilli occurring in cheese to the milk used for their making and to the air of the dairy. Several of the types found in the air were isolated some time before or after the cheese from which they were also isolated had been made, and some of these types were isolated from cheeses made on a number of different occasions (2). This strongly suggests a resident flora in the dairy or a reservoir of constant infection nearby. In either case such a stable lactobacillus flora would lead to a recurrence of types of lactobacilli in the cheeses which might influence the flavour considerably.

When the milk had a high lactobacillus count (approx. 200/ml.) the serological types from milk predominated in the cheese. When the counts were low the flora of the cheeses was more diverse and few, if any, of the cheese serological types were found in the original milk. In these cases contamination from the air and other sources would be more likely to influence the cheese flora.

The numbers of lactobacilli found in the air were extremely low, but nevertheless one type isolated from the air during cheese-making was subsequently found in the cheese in large numbers. It is very likely also that the cheese milk itself infects the air.

Most of the lactobacilli found in both milk and in air were low-temperature homofermentative strains which suggests contamination from sources such as a resident flora in the environs of the dairy or hay, silage or manure. *L. plantarum* was found in hay but none was of the same type as those found in cheese.

Large numbers of *L. casei* were found in saliva but these could not be traced to the cheese.

The numbers of lactobacilli found in milk were very different in summer and winter, but as the number of milks examined was small it cannot be ascertained until further work is done whether this was a seasonal or a chance variation. The small numbers of lactobacilli isolated from the earlier samples confirm the findings of Jensen & Edmondson (20), who isolated lactobacilli from only 95 out of 406 samples of milk and cream by enrichment methods. The influence of the milk flora on the cheese flora must be complex, as not only are the numbers and species of lactobacilli variable but other organisms capable of growth in cheese, such as leuconostocs and pediococci, can also occur. The origin of the lactobacilli in the milk itself was not discovered, but contamination from hay and manure would seem to be a likely source.

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734. FACTORS INFLUENCING THE VITAMIN CONTENT OF MILK FAT

VIII. THE INFLUENCE OF VARIOUS FACTORS ON THE UTILIZATION OF CAROTENE

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Marked seasonal variations have been found to occur in the levels of vitamin A and carotenoids in the blood plasma and milk fat of cows under typical New Zealand conditions of pasture feeding (1,2,3,4,5,6). These cannot be ascribed to marked changes in the carotene intake of the cows (4) and must be due to other changes, either environmental (7) or in the composition of the pasture (8). Factors influencing carotene utilization have been investigated by a number of workers, but in general the level of carotene intake has been relatively low and it cannot be assumed that the same effects would be apparent at the consistently higher levels of carotene intake obtaining with cows on pasture in this country (4–8 g./cow/day) (4).

Little information is available about variations in the composition of typical New Zealand pasture. It is well known, however, that there are seasonal changes in the botanical composition of the pasture, particularly in the proportion of rye grass (*Lolium perenne*) and red and white clover (*Trifolium pratense* and *repens*) (9), and also in the stage of maturity. Variations in known pasture constituents associated with these herbage changes might markedly affect carotene utilization and as a possible guide to future experiments using cows it was felt that useful information could be obtained in the first place using rats as experimental animals. It was fully realized that the different physiological make-up of the cow and the rat, particularly the wide differences in their digestive systems, was a complicating factor, and that the oils used as a carrier for carotene were not of the same composition as the oils naturally present in pasture herbage. Bearing in mind these limitations the following variables which might affect carotene utilization were therefore investigated with rats.

(*a*) *Effect of level of carotene.* Although the carotene intake of the cows is relatively constant throughout the year, small fluctuations about the average high level do occur.

(*b*) *Effect of tocopherol.* Differences in the tocopherol content of typical pastures have already been noted (8).

(*c*) *Effect of chlorophyll and xanthophyll.*

(*d*) *Effect of concentration of carotene* in the oil used as carrier.

(*e*) *Effect of degree of unsaturation of the carrier fat.* Although pasture fats are highly unsaturated (10), a considerable amount of hydrogenation may occur in the rumen (11).

(*f*) *Effect of non-digestible material in the carrier.*

(*g*) *Effect of botanical composition of the feed.*

* On leave from the National Institute for Research in Dairying, Shinfield, Reading, England.

(*h*) *Effect of cyanide.* The white clover in New Zealand pastures has a high content of cyanogenetic glucoside equivalent to about 0·03% cyanide on a dry-matter basis (12).

In addition, since in cows the seasonal changes, particularly in the plasma vitamin A alcohol levels could be due to hormonal influences, the effects of adrenalin and insulin which other workers have claimed could influence plasma vitamin A levels (13, 14) were also investigated.

EXPERIMENTAL

Sources of materials used for feeding or injection

Carotene. Crystalline carotene was obtained from Eastman Kodak Co., Ltd. It contained some 90% β-carotene and 10% α-carotene. Solutions for feeding were prepared in arachis oil as described by Thompson, Ganguly & Kon (15).

Tocopherol. α-Tocopheryl acetate was supplied by Eastman Kodak Co., Ltd.

Triolein. This material was supplied by Dr F. B. Shorland, Fats Research Laboratory, Department of Scientific and Industrial Research, Wellington, New Zealand.

Tristearin and beeswax (white). These substances were obtained from the British Drug Houses, Ltd., Poole, Dorset.

Xanthophylls and chlorophyll. Fresh grass was extracted in the cold with acetone and the pigments were separated by chromatography on aluminium oxide as described by Worker (16).

Rye grass and clovers. Rye grass (*Lolium perenne*), white clover (*Trifolium repens*) high in cyanogenetic glucoside (equivalent to about 0·03% cyanide on a dry-matter basis) or white clover low in cyanogenetic glucoside, were freeze dried in an apparatus made at Massey College and then milled to pass through a 1 mm. sieve.

Adrenalin and insulin. Adrenalin 1:1000 was manufactured by Kempthorne & Prosser (N.Z.) Ltd. and Insulin 'A.B.' 80 i.u./ml. was as manufactured by Allen and Hanburys, Ltd. and British Drug Houses, Ltd.

Preparation and dosing of rats

The rats used were albinos of the Wistar strain rendered partially deficient in vitamin A as described by McGillivray, Thompson & Worker (17). For the hormone work stock colony rats with normal vitamin A reserves in the liver were used.

Unless otherwise stated, the carotene and other fat-soluble substances were dissolved in arachis oil and the animals dosed by stomach tube and killed 48 hr. later.

The amount of freeze-dried herbage fed was adjusted according to its carotene content to give the same intake of carotene, and the herbage was given mixed with skim-milk powder. The rats were offered each day no more than they would consume in 24 hr.

Potassium cyanide was added either to the drinking water to give a concentration of 0·05% as HCN or was mixed in the diet at the rate of 25 mg./rat/day. The experimental feeding lasted 6 days.

Adrenalin or insulin were administered subcutaneously as 0·5 ml. to stock colony rats which were killed ½ hr. later.

Measurement of vitamin A, carotenoids and tocopherol

Vitamin A and carotenoids were determined in the blood plasma and liver as described by Thompson *et al.* (15). Liver tocopherol was estimated by a method described by Worker (18).

RESULTS

The results presented in Table 1 show that several possible pasture variables might be expected to affect quite markedly carotene utilization by cows despite the high level fed. Thus from Exp. 1 it is clear that a fairly direct relationship exists between liver storage of vitamin A and the level of carotene fed over the range of 0·25 and 4 mg./rat. The concentration of carotene in the oil is also important (Exp. 2), too low or too high a concentration tending to reduce liver storage, the latter owing probably to an overloading of the digestive system with lipid material. From Exp. 3 it is apparent that the nature of the vehicle may have a profound effect on carotene utilization. When carotene was dissolved in mixtures of triolein and tristearin the highest liver stores of vitamin A were obtained with either pure triolein or at least 70% of triolein. The incorporation of a little beeswax, a typical hard wax which might compare with leaf cuticle wax, had an obvious depressing effect, as a mixture of 60% triolein, 25% tristearin and 15% beeswax resulted in a liver storage of only 8·3 μg. vitamin A, whereas a mixture of 60% triolein and 40% tristearin alone would have been expected to give liver stores of the order of 40–45 μg. vitamin A. Variation in the level of tocopherol from 0 to 8 mg. had no effect in the utilization of the 4 mg. dose of carotene (Exp. 4). Similarly, addition of 40 mg. chlorophyll to the carotene had no effect (Exp. 5), whereas 3 mg. xanthophyll depressed liver vitamin A stores slightly, probably not significantly. In general, liver carotene levels, although low, followed the liver vitamin A levels. Plasma vitamin A alcohol levels varied from 13 to 31 μg./100 ml. plasma and, as would be expected, bore no apparent relationship to treatment. On the other hand, plasma ester values, which ranged from 1·1 to 8·6 μg./100 ml., bore some relationship to liver vitamin A levels, indicating that absorption of vitamin A was still proceeding at 48 hr. after dosing.

From the results presented in Table 2 it would appear that there was some difference in the utilization of carotene by rats from the three pasture species investigated, but the addition of cyanide in quantities equivalent to those likely to be derived from a 'high cyanide' clover had no very marked effect on the utilization of carotene either from arachis oil or from a mixed pasture. In Exp. 1, the lowest liver storage of vitamin A resulted from feeding 'high cyanide' clover. The plasma vitamin A alcohol level of 34 μg./100 ml. may be compared with that of 20 and 26 μg. for the rye grass and 'low cyanide' clover, respectively. The same effect is apparent in Exp. 2, where the addition of cyanide to the drinking water resulted in lower liver storage of vitamin A again associated with higher blood plasma alcohol levels. However, the addition of KCN to the normal vitamin A-deficient diet supplemented with carotene in oil was without effect on plasma or liver levels of vitamin A.

To investigate the effect of adrenalin or insulin on plasma vitamin A levels, female stock colony rats were injected simultaneously with 0·5 ml. of either 1:1000 adrenalin or 40 i.u. of insulin and killed $\frac{1}{2}$ hr later. Six similar rats injected with 0·5 ml. distilled water were used as controls. From the results (Table 3, Exp. A) it can be seen that the plasma vitamin A alcohol levels in the adrenalin-treated animals (11·3 μg./100 ml.) were not significantly lower than those for the control animals (15·1 μg./100 ml.). When this experiment was repeated with animals with considerably higher liver vitamin A reserves (Exp. B) similar injections of adrenalin produced again no significant changes in plasma vitamin A alcohol, six treated animals averaging 14·4 μg./100 ml. and six control animals

Table 1. *The effect of various factors on the appearance of vitamin A and carotene in the blood plasma and livers of female rats (mean weight 250 g.) partially deficient in vitamin A dosed orally with carotene. All rats were slaughtered 48 hr. after dosing*

		Dose				Blood plasma, vitamin A		Liver				
		Vehicle			Other substances			Vitamin A				
Exp. no.	No. rats used	Nature	Amount (g.)	Amount carotene (mg.)	Nature	Amount (mg.)	Alcohol (μg./100 ml.)	Ester (μg./100 ml.)	Alcohol (μg.)	Ester (μg.)	Total (μg.)	Carotene (μg.)	Tocopherol (μg.)
	6	—	—	—	—	—	13	—	0·5	0·7	1·2	—	—
1	3	Arachis oil	1	0·25	—	—	18	1·1	1·0	2·3	3·3	1·8	—
	3		1	0·5	—	—	16	1·5	2·2	4·9	7·1	1·9	—
	3		1	1·0	—	—	19	3·3	3·8	8·2	12	2·3	—
	3		1	2·0	—	—	20	4·0	9·1	19	28	5·3	—
	3		1	4·0	—	—	24	7·0	10	38	48	8·7	—
2	3	Arachis oil	0·5	4·0	—	—	25	5·2	14	24	38	6·7	—
	3		1·0	4·0	—	—	24	7·0	10	38	48	8·7	—
	3		2·0	4·0	—	—	31	3·3	10	16	26	4·8	—
3	3	30% triolein + 70% tristearin	1	4·0	—	—	19	2·8	2·1	3·7	5·8	5·4	—
	3	40% triolein + 60% tristearin	1	4·0	—	—	19	3·7	6·3	19	25	8·2	—
	3	50% triolein + 50% tristearin	1	4·0	—	—	20	3·9	9·8	26	36	13	—
	3	70% triolein + 30% tristearin	1	4·0	—	—	16	5·7	11	38	49	10	—
	3	Triolein	1	4·0	—	—	18	5·2	12	40	52	11	—
4	3	60% triolein + 25% tristearin + 15% beeswax	1	4·0	—	—	17	3·1	2·5	5·8	8·3	6·3	—
4	3	Arachis oil	1	4·0	α-tocopheryl acetate	0	24	7·0	10	38	48	8·7	13
	3		1	4·0		0·5	25	4·2	11	30	41	8·1	17
	3		1	4·0		1·0	20	5·0	11	38	49	8·3	18
	3		1	4·0		2·0	21	8·6	11	38	49	11	20
	3		1	4·0		4·0	25	2·9	10	32	42	5·6	30
	3		1	4·0		8·0	17	2·2	11	33	44	4·8	42
5	3	Arachis oil	1	4·0	—	—	24	7·0	10	38	48	8·7	—
	3		1	4·0	Chlorophyll	40	25	2·3	14	33	47	4·1	—
	3		1	4·0	Xanthophyll	3	25	3·0	12	26	38	4·6	—
	3		1	4·0	Chlorophyll + Xanthophyll	40 + 3	25	3·4	13	30	43	4·7	—

Table 2. *The effect of feeding separately ryegrass, 'low cyanide' and 'high cyanide' white clover and of cyanide on the appearance of vitamin A and carotene in the blood plasma and liver of male rats (mean weight 150–190 g.) partially deficient in vitamin A*

		Treatment			Blood plasma vitamin A		Liver				
							Vitamin A			Carotenoids	
Exp. no.	No. rats used	Diet	Duration days	Total carotene intake per rat (mg.)	Alcohol (μg./100 ml.)	Ester (μg./100 ml.)	Alcohol (μg.)	Ester (μg.)	Total (μg.)	Carotene (μg.)	Xantho-phyll (μg.)
		Controls	—	—	—	—	—	—	—	—	—
I	6	Normal vitamin A-deficient diet	—	—	14	—	0·5	0·4	0·9	0	0
	6	Skim-milk powder + 28% freeze-dried rye grass	6	7·1	20	7·6	24	82	106	2·8	5·3
	6	Skim-milk powder + 34% freeze-dried 'low cyanide' white clover	6	7·1	25	8·2	21	73	94	2·6	2·6
	6	Skim-milk powder + 50% freeze-dried 'high cyanide' white clover	6	7·1	34	8·8	20	60	80	3·2	3·0
II	4	Skim-milk powder + 30% freeze-dried pasture (approx. 50% rye grass 50% 'low cyanide' clover on dry-matter basis)	5	6·6	27	—	15	113	128	6·2	4·3
	4	Skim-milk powder + KCN equivalent to 0·05% HCN in drinking water	5	6·6	33	—	20	91	111	6·9	3·5
III	3	Normal vitamin A-deficient diet + 4 mg. carotene in 400 mg. arachis oil/rat/day	6	24	22	—	23	310	333	34	—
	3	Normal vitamin A-deficient diet + KCN equivalent to 25 mg. HCN/rat/day mixed with feed		24	22	—	28	334	362	34	—

14·6 μg./100 ml. The injection of insulin was also found to have no effect on plasma alcohol. The level of 13·2 μg./100 ml. was not significantly different from the values for the controls.

Table 3. *The effect of subcutaneously administered adrenalin and insulin on the plasma vitamin A alcohol levels of female stock colony rats. Slaughtered ½ hr. after injection. Mean weight* 300 *g.*

	No. of rats	Liver reserves of vitamin A per rat (μg.)	Dose	Blood plasma vitamin A alcohol (μg./100 ml.)
Exp. A	6	Normal 100–250	0·5 ml. 1:1000 adrenalin	11·3
	6	Normal 100–250	0·5 ml. distilled water	15·1
Exp. B	6	High 2500	0·5 ml. 1:1000 adrenalin	14·4
	6	High 2500	0·5 ml. insulin = 40 u.	13·2
	6	High 2500	0·5 ml. distilled water	14·6

DISCUSSION

Factors influencing carotene utilization at low levels of carotene intake have been extensively reviewed by a number of workers (see, for example, Goodwin (19)), and will not be dealt with further here. The main object of our experiments was to determine whether the same factors operate at considerably higher levels of carotene intake, and the results may serve to explain certain irregularities in the utilization of carotene from pasture by dairy cows grazing under New Zealand summer conditions (1,2,3,4,5,6). For this purpose rats were used as experimental animals and all dosage rates were adjusted, on a body-weight basis, so as to simulate as closely as possible the conditions which would obtain in the grazing cow.

From the results obtained it is apparent that certain factors do have a profound influence on the vitamin A economy of the animal even at these relatively high levels of carotene intake. For example, it is clear from our results that over a wide range of carotene intake there is an almost linear relationship between hepatic storage of vitamin A and level of intake. Similarly, there would appear to be a relationship between the level of fat in the diet and the efficiency of carotene utilization provided the digestive system does not become unduly overloaded with lipid material which may seriously impair utilization. In contrast to these results, however, it would appear that the utilization of carotene when fed at high levels in the diet is but little affected by tocopherol, xanthophyll or chlorophyll. From a consideration of the results obtained in Exp. 3 (Table 1) it is apparent that the degree of unsaturation of the oily vehicle may be the most important single factor determining the efficiency of utilization of carotene at high intake levels. In practice in the field the degree of unsaturation of the pasture fat could affect the utilization of carotene in at least two ways: directly owing to the proportion of unsaturated fatty acids present in the fat, and indirectly depending on the extent to which the unsaturated acids present are hydrogenated by microbial activity during their passage

through the rumen. Obviously, further work will be required to elucidate these relationships more clearly.

From the results of the second series of experiments, in which the effect was studied of feeding separately rye grass, 'low cyanide' and 'high cyanide' white clover on plasma levels and hepatic stores of vitamin A, a difference was observed in the utilization of carotene from the three species in favour of the rye grass and 'low cyanide' clover (Exp. A). This species difference was, however, probably due to some factor other than the cyanide content, since the incorporation of cyanide in the diet in amounts calculated to be equivalent to those derived from 'high cyanide' clover did not appear to affect appreciably the efficiency of utilization of carotene either from a mixed pasture or from arachis oil.

It appears that under the conditions of our experiment neither adrenalin nor insulin had any appreciable effect on the levels of vitamin A alcohol in the blood plasma of rats. These results are in sharp contrast to the findings of Young & Wald (13) and Bauereisen (14).

SUMMARY

1. The influence of a number of factors on the utilization of carotene by rats has been investigated. A high dose level equivalent to that derived from pasture by cows has been employed and liver storage of vitamin A has been used as a measure of the efficiency of carotene utilization.

2. Utilization was influenced by level of carotene intake, by concentration of carotene in the oil used as vehicle, by the degree of unsaturation of the vehicle and by the presence of non-digestible wax.

3. Carotene was better utilized from the rye grass and 'low cyanide' white clover than from 'high cyanide' clover, but added cyanide had little or no effect on the uptake of carotene from cyanide-free pasture or from a solution in oil.

4. In so far as the results of the investigation can be applied to cows grazing typical New Zealand dairy pasture, it seems that factors which may throw some light on the poor utilization of carotene at certain times of the year are (*a*) small day-to-day variation in the carotene content of the pasture, (*b*) variations in the 'ether extract' fraction of the pasture, and (*c*) variations in the degree of hydrogenation of the fat in the rumen.

5. Insulin and adrenalin appear to have no influence on the levels of vitamin A alcohol in the blood plasma of rats.

The results of this investigation form a section of a thesis submitted by one of us (N.A.W.) in partial fulfilment of the requirements for the degree of Ph.D. of the University of New Zealand. The authors are indebted to the Department of Scientific and Industrial Research for a Grant towards this investigation and to Miss Fay Frecklington for technical assistance; one of us (S.Y.T.) is indebted to the Royal Society, the Nuffield Foundation, and the Board of the Dairy Research Institute (N.Z.) for a travelling fellowship during the tenure of which the work reported here was carried out.

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735. THE B-VITAMIN CONTENT OF MILK IN RELATION TO BREED OF COW AND STAGE OF LACTATION*

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(With 2 Figures)

Houston, Kon & Thompson (2) used chemical methods to study the effect of stage of lactation and of season on the thiamine and riboflavin content of cow's milk. Using microbiological methods, we have extended their early work to include biotin, nicotinic acid, pantothenic acid, vitamin B_6 and vitamin B_{12}.

With cows maintained on the Institute farm under normal conditions of good herd management we have compared (*a*) the day-to-day variation in the concentrations of these vitamins in milk from individual cows of three breeds (Shorthorn, Friesian and Guernsey), and (*b*) the concentrations of these vitamins in samples of milk taken at intervals throughout lactation from Friesian and Shorthorn cows. In these experiments we were able to study the variation in the B-vitamin content of milk in relation to breed of cow and stage of lactation. It was not possible to consider independently the effects of season and of stage of lactation upon the milk vitamin levels. Over half the cows used in the experiment calved in October and November.

EXPERIMENTAL

Collection of samples

(*a*) For the measurement of day-to-day variation in the B-vitamin content of milk, samples from the morning and afternoon milkings were collected from eleven cows in mid-lactation (three Guernsey, four Shorthorn and four Friesian) on 7 consecutive days in May 1952. The Guernsey cows were grazing on cocksfoot and clover sward and the Friesian and Shorthorn cows on white clover and rye grass. None of the cows received concentrates during this experiment. The morning and afternoon milks from each cow were mixed in proportion to their yields.

(*b*) For the study of lactational trends in the B-vitamin content of milk, ten Shorthorn and ten Friesian cows, that calved between 21 July 1952 and 18 January 1953 were used. From October until April the cows were maintained on a normal farm diet of concentrates, with kale or silage and hay. From April until October they were on good pasture with additional concentrates given to the high-yielding cows. Samples were taken from each cow at morning and afternoon milkings on the first 4 days after calving, then on the 7th, 14th and 28th days, and thereafter at 28-day intervals until the end of lactation. The morning and afternoon milk samples taken on the first 4 days were analysed separately, but subsequently the morning and afternoon milks were mixed in proportion to their yields.

* Presented in part at the XIVth International Dairy Congress (1).

All samples were dispensed in several 5 ml. portions into screw-stoppered tubes and stored at −20° C. until assayed.

Microbiological assays

General. In preliminary experiments we have established the optimum conditions for the measurement of biotin, nicotinic acid, pantothenic acid, riboflavin, vitamin B_6 and vitamin B_{12} in cow's milk. It should be pointed out that these methods are not necessarily the best for the milk of other species. For example, sow's milk and human milk require a more drastic treatment than the one described here for releasing vitamin B_{12} from cow's milk (3).

Several of the vitamins were fully available to the test micro-organisms without any extraction, i.e. whole milk could be simply diluted with water and added to the assay tubes. However, we preferred to adjust the milk extract to pH 4·6 and remove the precipitated proteins and occluded fat by filtration before adding the extracts to the assay tubes. This procedure has two advantages: (*a*) it gives a clear extract which enables growth responses to be measured turbidimetrically, and (*b*) it removes the fat which can cause interference in the tests. The microbiological methods finally adopted were as follows.

Biotin and nicotinic acid

(*a*) *Treatment of samples.* The milk samples (2 ml.) were autoclaved with 10 ml. of 0·2 N-H_2SO_4 at 10 lb. pressure for 30 min. The extracts were cooled, diluted to 50 ml., adjusted to pH 4·6 and filtered. The filtrate was adjusted to pH 6·8 and diluted to contain about 0·05 μg. of nicotinic acid/ml. for the nicotinic acid assay, and then further diluted for biotin assay to about 0·0001 μg. of biotin/ml., these levels being determined approximately in preliminary tests.

(*b*) *Method of assay.* The test organism was *Lactobacillus arabinosus* 17–5 grown in the medium of Roberts & Snell (4), modified by reducing the amounts of KH_2PO_4 and K_2HPO_4 to 0·5 g. of each/l. of double-strength medium and by adding riboflavin and omitting the particular vitamin under test. Sterilization was by heating in steam at 10 lb. pressure for 10 min. The inoculum was prepared by growing the test organism for 24 hr. in 10 ml. of basal medium containing limiting amounts of the vitamin under test. The 24 hr. culture was diluted 1:100 in 0·9% saline and one drop of this suspension used to inoculate each assay tube.

Incubation was for 48 hr. at 37° C. in a constant-circulation water-bath and growth was measured turbidimetrically in a Lumetron Model 400A colorimeter.

Pantothenic acid

(*a*) *Treatment of samples.* In preliminary tests, milk samples were treated with intestinal phosphatase and a chicken-liver enzyme as recommended by Neilands & Strong (5) for the extraction of pantothenic acid from natural materials. This treatment gave results about 10% higher than those obtained without the enzymes, but the high pantothenic acid blank of the chick-liver enzyme made them less precise. Further tests showed that the pantothenic acid activity of milk was largely free, since it was able to pass into protein-free ultrafiltrates of the milk (6). For these reasons we have found it satisfactory to measure pantothenic acid as follows. The milk (1 ml.), diluted with 5 ml. of distilled water, was heated in steam for 10 min. After cooling, 0·2 ml. of 2·5 M-sodium acetate buffer

(pH 4·6) was added and the pH of the milk adjusted to 4·6 at a volume of 100 ml. The extract was filtered and the filtrate diluted to contain about 0·04 μg. pantothenic acid/ml. The reference standard was a solution containing 0·04 μg. Ca pantothenate/ml.

(*b*) *Method of assay.* It was the same as that described for biotin and nicotinic acid.

Riboflavin

(*a*) *Treatment of samples.* The milk (1 ml.) was autoclaved at 15 lb. pressure for 15 min. in 20 ml. of 0·1 N-HCl as recommended by the Analytical Methods Committee, Society of Public Analysts (7). The extract was filtered at pH 4·6 and diluted to contain about 0·01 μg. riboflavin/ml.

(*b*) *Method of assay.* Riboflavin was measured with *Lactobacillus casei* ATCC 7469 in the medium of Skeggs, Nepple, Valentik, Huff & Wright (8) modified by omitting riboflavin, thiomalic acid and ribonucleic acid.

'*Vitamin* B_6'

(*a*) *Treatment of samples.* The milk (1 ml.) was autoclaved with 90 ml. of 0·055 N-H_2SO_4 at 20 lb. pressure for 5 hr., as recommended by Rabinowitz & Snell (9). The extracts were diluted to 100 ml., adjusted to pH 4·6, filtered and diluted further to contain about 0·01 μg. 'vitamin B_6'/ml.

(*b*) *Method of assay.* The method was that of Atkin, Schultz, Williams & Frey (10) modified as recommended by Hopkins & Pennington (11). The test organism was *Saccharomyces carlsbergensis* 4228, which under our conditions of test responds about equally to pyridoxal, pyridoxamine and pyridoxine. Vitamin B_6 activity is expressed in terms of a standard of pyridoxine hydrochloride.

Vitamin B_{12}

(*a*) *Treatment of samples.* The milk samples were prepared for assay by treatment with cyanide at pH 4·6 as described by Gregory (3).

(*b*) *Method of assay.* Vitamin B_{12} was measured with *Lactobacillus leichmannii* ATCC 4797 in the medium of Skeggs *et al.* (8) as described by Gregory (3).

Statistical design of the assays for measuring lactational trends in the B-vitamin content of milk

When all the samples from the twenty cows had been collected, assays were done so that samples from a Shorthorn and a Friesian cow were examined simultaneously. In order to compare the levels in milk from individual cows and to establish any breed differences, the tubes containing the first eight samples from each cow were arranged in the same basket. The second basket contained the next eight samples of each and the third basket the end-of-lactation samples from each cow. Each basket included tubes containing a standard of the vitamin under test, set up (in duplicate) at levels of 0, 1·0, 2·0, 3·0 and 4·0 ml. of solution of appropriate concentration. The milk extracts were added at levels of 1·0, 2·0 and 4·0 ml. The tubes in each basket were randomized before sterilization and inoculation to prevent bias due to the position of the tubes in the autoclave or incubator. At least two assays were done for each vitamin.

The results were calculated by comparing the growth response of the test organism to

the test preparation with that to the standard vitamin solution, by means of a nomogram for the rapid estimation of potencies and limits of error (12).

Chemical analyses. All milk samples from each cow were analysed for total solids and fat.

RESULTS

Variations from day-to-day and between cows of the same and different breeds

The mean daily levels, with standard deviations, calculated over the 7-day test period for six vitamins in the milk from three Guernsey, four Shorthorn and four Friesian cows are shown in Table 1. The levels of each vitamin varied between cows of the same breed, and from day-to-day in milk samples from individual cows.

Table 1. *Mean values with their standard errors* (S.E.) *for biotin, nicotinic acid, pantothenic acid, riboflavin, 'vitamin B_6' and vitamin B_{12} in milk of individual Guernsey, Friesian and Shorthorn cows sampled on 7 consecutive days*

	Biotin (mμg./ml.)		Nicotinic acid (μg./ml.)		Pantothenic acid* (μg./ml.)		Riboflavin (μg./ml.)		'Vitamin B_6'† (μg./ml.)		Vitamin B_{12} (mμg./ml.)	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Guernsey												
Charis	9	2	0·58	0·14	2·4	0·2	1·8	0·2	0·26	0·04	3·2	1·2
Dido	8	3	0·55	0·04	3·3	0·6	1·6	0·4	0·17	0·05	2·1	0·4
Pandora	11	3	0·71	0·07	4·3	0·5	1·7	0·2	0·24	0·04	2·9	0·8
Friesian												
Annabelle	13	4	1·31	0·11	3·5	0·4	1·1	0·1	0·25	0·03	2·5	0·7
Marian	21	4	0·77	0·09	5·3	0·3	1·4	0·1	0·29	0·03	2·3	0·7
Petunia	19	4	0·88	0·13	5·3	0·6	1·4	0·2	0·26	0·03	3·3	0·7
Pollyanna	21	3	0·80	0·11	5·5	0·5	1·3	0·3	0·23	0·02	3·9	0·5
Shorthorn												
Buttercup	14	2	0·64	0·13	2·8	0·2	1·1	0·2	0·22	0·04	2·4	0·2
Clara	16	3	0·67	0·13	4·4	0·4	1·0	0·1	0·24	0·03	2·2	0·2
Rosalie	19	3	0·45	0·04	3·5	0·0	1·1	0·1	0·21	0·03	1·6	0·3
Winnie	21	3	0·57	0·07	5·3	1·1	1·1	0·2	0·36	0·03	2·0	0·3

* Measured as Ca-D-pantothenate. † Measured as pyridoxine hydrochloride.

Variation with stage of lactation.

The variations in biotin, nicotinic acid, pantothenic acid, riboflavin, vitamin B_6 and vitamin B_{12} content with stage of lactation in milk from Shorthorn and Friesian cows are shown in Figs. 1 and 2, as mean values at the chosen intervals throughout lactation. The standard error of certain of the mean values is included as an indication of the significance of the curves. In the later stages of lactation, the number of samples (shown in parentheses) represented by each point became smaller because of early drying-off or loss from the herd of some of the cows. Table 2 shows the mean levels of the vitamins in milk from individual cows for the period from the 1st to the 6th month of lactation and typical values for bulk winter milk of the herd.

The biotin content of the first samples of colostrum ranged from 3 to 28 mμg./ml. for Friesians and from 5 to 31 mμg./ml. for Shorthorns. The level increased to a maximum on about the 4th day of lactation (38–71 mμg./ml. for Friesians; 28–76 mμg./ml. for Shorthorns) and then fell rapidly during the next 3 or 4 days. The levels in the milk varied considerably from cow to cow.

The range of values for nicotinic acid in colostrum (0·52–1·09 μg./ml. for Friesians and 0·51–1·00 μg./ml. for Shorthorns) was similar to the range in the milk (0·28–1·01 μg./ml. for Friesians and 0·25–0·79 μg./ml. for Shorthorns). However, the mean values for each breed indicated a higher level of nicotinic acid in colostrum. This mean level decreased gradually up to the 4th or 5th month and then increased gradually until the end of lactation.

The levels of pantothenic acid were low in the first samples of colostrum and ranged from 0·3 to 3·6 μg./ml. for Friesians and 0·8–2·3 μg./ml. for Shorthorns. The values rose to

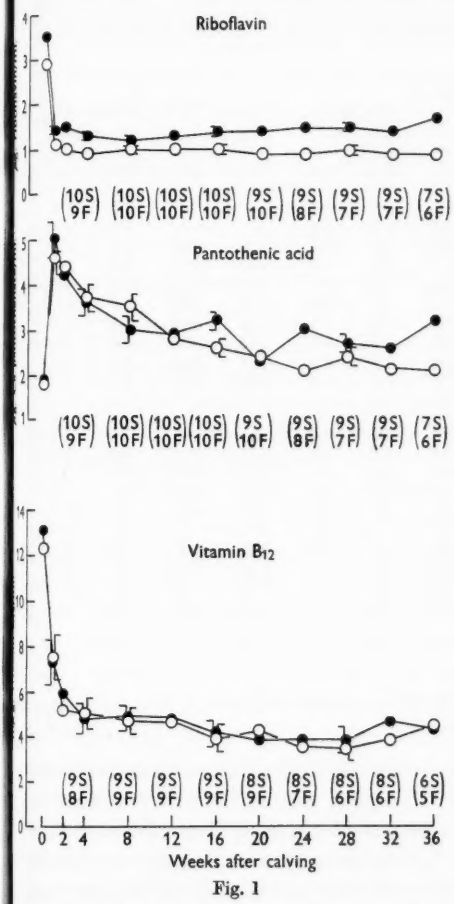

Fig. 1

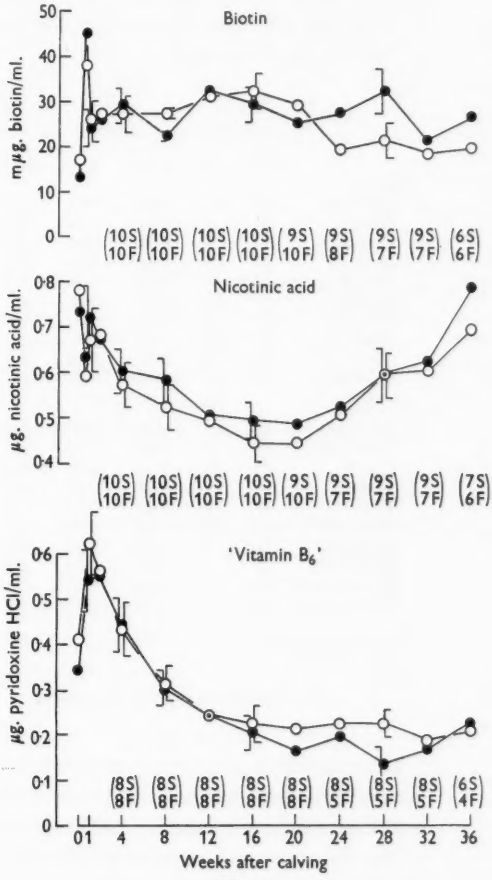

Fig. 2

Fig. 1. Variation in riboflavin, pantothenic acid and vitamin B_{12} contents with stage of lactation in milk from Shorthorn (o—o) and Friesian (●—●) cows. The numbers in parentheses indicate the number of cows represented. Symbols o[give the standard errors of the means for Shorthorn cows and symbols]● those for Friesian cows.

Fig. 2. Variation in biotin, nicotinic acid and vitamin B_6 contents with stage of lactation in milk from Shorthorn (o—o) and Friesian (●—●) cows. The numbers in parentheses indicate the number of cows represented. Symbols o[give the standard errors of the means for Shorthorn cows and symbols]● those for Friesian cows.

a maximum on the 4th to 14th day of lactation when the range was 3·6–8·8 μg./ml. for Friesians and 4·1–6·6 μg./ml. for Shorthorns. The level then fell gradually to about the 20th week.

Riboflavin was high in the first sample of colostrum (2·3–6·6 μg./ml. for Friesians and 2·3–6·7 μg./ml. for Shorthorns). This level fell rapidly and had reached the normal milk level by the end of the first week. The milk of the Shorthorn cows was significantly lower in riboflavin than the milk of the Friesians.

Table 2. *Mean values with their standard errors* (S.E.) *for vitamin levels in the milk of Friesian and Shorthorn cows.* (*Samples were taken at 4-week intervals from the 1st to 6th month of lactation*)

	Biotin (mμg./ml.)		Nicotinic acid (μg./ml.)		Pantothenic acid* (μg./ml.)		Riboflavin (μg./ml.)		'Vitamin B_6'† (μg./ml.)		Vitamin B_{12} (mμg./ml.)	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Friesian												
Adelaide	26	18	0·47	0·09	2·9	0·7	0·9	0·1	—	—	3·6	1·9
Hilary	21	5	0·74	0·18	2·9	0·5	1·3	0·1	—	—	—	—
Humbug	23	5	0·34	0·06	2·8	0·6	1·3	0·1	0·29	0·09	3·8	0·9
Lead 29	27	12	0·46	0·11	2·2	0·7	1·3	0·2	0·31	0·18	5·7	2·4
Maid 2	27	6	0·55	0·19	3·4	1·2	1·4	0·2	0·13	0·11	5·4	1·2
Marion	32	15	0·45	0·08	4·3	1·1	1·1	0·2	0·13	0·08	2·9	0·7
Mirlan	21	8	0·56	0·07	1·5	0·3	1·1	0·1	0·14	0·05	3·7	0·7
Ocarina	43	18	0·61	0·03	3·1	0·6	1·5	0·3	0·43	0·15	5·0	1·1
Opera	23	9	0·54	0·12	2·2	1·4	1·8	0·4	0·33	0·12	5·0	1·7
Petunia	30	5	0·55	0·02	4·3	0·7	1·5	0·4	0·34	0·13	4·0	1·7
Shorthorn												
Flora 104	40	12	0·42	0·12	3·2	0·4	1·5	0·5	—	—	7·9	1·3
Flora 108	28	8	0·39	0·10	2·5	0·5	0·9	0·2	0·31	0·06	4·3	2·0
Ivy 4	26	7	0·45	0·09	3·0	0·7	0·8	0·1	0·31	0·11	5·7	2·4
Pearl 13	22	8	0·48	0·07	3·0	1·1	0·7	0·1	0·29	0·13	3·3	1·6
Phoebe 7	34	11	0·40	0·13	2·9	1·3	1·1	0·1	0·35	0·17	2·9	0·7
Pippin 15	20	8	0·67	0·09	2·7	0·7	1·2	0·2	—	—	—	—
Pippin 18	26	6	0·50	0·10	2·7	0·5	1·3	0·2	0·09	0·08	7·2	0·8
Rosalie 39	25	6	0·58	0·09	3·5	0·6	0·7	0·1	0·33	0·03	3·1	0·7
Winsome 26	31	17	0·43	0·16	2·4	0·7	0·7	0·1	0·13	0·07	2·1	0·6
Winsome 29	36	9	0·58	0·13	2·8	0·7	0·9	0·1	0·35	0·11	3·3	0·8
Bulk winter milk from N.I.R.D. herd	22		0·67		3·7		1·2		0·31		3·6	

* Measured as Ca-D-pantothenate. † Measured as pyridoxine hydrochloride.

The vitamin B_{12} lactation curve was similar to the riboflavin one, in that the mean vitamin B_{12} level was high in colostrum and fell rapidly during the first few days of lactation, reaching a 'normal' milk level about the 2nd week of lactation. However, four of the Friesians and three of the Shorthorns had between 15 and 30 mμg. of vitamin B_{12}/ml. in the first sample of colostrum; three Friesians and four Shorthorns had between 5 and 15 mμg./ml. and the other two Friesians and two Shorthorns had less than 5 mμg./ml. The 'normal' milk level was also very variable both between cows of the same breed and in individual cows from one month to the next.

The level of 'vitamin B_6' in both Friesian and Shorthorn milk rose gradually up to the 7th to 14th day of lactation reaching a maximum of 0·41–0·70 μg./ml. for Friesians and 0·37–0·99 μg./ml. for Shorthorns. It then fell steadily to about the 5th month of lactation.

Other factors affecting the B-vitamin content of milk

We could find no effect of the number of previous lactations of each cow on the vitamin levels in the milk. The length of dry period appeared to have some effect on the 'vitamin B_6' and the riboflavin content of the milk during the 1st week of lactation. The longer the dry period, the lower were the levels of these two vitamins.

Although our experiments were not designed to measure seasonal variations there was a suggestion of a change in certain vitamin levels when the cows went out to grass. The levels of biotin and vitamin B_{12} dropped and that of nicotinic acid rose. However, no definite conclusions could be drawn, since milk samples from individual cows were taken at regular 4-week intervals from the date of calving, and therefore some cows were due for sampling after only a few days on pasture, whereas others were on grass for a period of up to 4 weeks before the milk sample was taken.

We could not find any correlation between the B-vitamin levels and the fat or solids-not-fat content of milk from individual cows.

DISCUSSION

There are many published values for the content of B-vitamins in commercial milk and in milk from different breeds of cow, but few systematic studies of the trends in the content of milk during lactation have been published. Contributions to this subject have been made by Houston *et al.* (2) for riboflavin and thiamine, by Lawrence, Herrington & Maynard (13) for pantothenic acid, nicotinic acid and biotin, by Anthony, Couch, Rupel, Henderson & Brown (14) for vitamin B_{12} and by Collins, Boldt, Elvehjem & Hart (15) for vitamin B_{12} and folic acid.

Our measurements of the content of B-vitamins in milk taken daily from individual cows over a period of 7 days, showed that a day-to-day variation of at least 10% can be expected in the levels of biotin, nicotinic acid, riboflavin, pantothenic acid, 'vitamin B_6' and vitamin B_{12} (see Table 1). With biotin and vitamin B_{12} the variation was often greater. Both these vitamins are present in milk in smaller quantities than the other vitamins. Thus a Friesian cow with a daily milk yield of 37 lb. would secrete per day about 80 mg. of pantothenic acid, 20 mg. of riboflavin, 15 mg. of nicotinic acid and 5 mg. of 'vitamin B_6', as compared with 300 μg. of biotin and 50 μg. of vitamin B_{12}. The fluctuations from one day to the next do not arise from errors in the method of assay, since duplicate assays set up on different days were in excellent agreement. Similar variations have been reported by Lawrence *et al.* (13) who found an average difference from one day to the next of 7% for nicotinic acid and 10% for pantothenic acid. They report the biotin fluctuations as being especially wide and quite unpredictable.

In addition to day-to-day variations we also found variations in the levels of any one of the vitamins in milk from cows of the same breed (see Tables 1 and 2). It can be seen from Figs. 1 and 2 that, with the exception of the riboflavin level, there was no significant difference between Shorthorn and Friesian milk.

The results of our study of changes in the content of B-vitamins in milk throughout lactation are generally in agreement with those obtained by other workers (2, 13, 14, 15).

The variations in biotin content—low in early colostrum, rising to a maximum on the 4th to 7th day, and then decreasing rapidly to the 'normal' milk level—were very similar

to those found by Lawrence *et al.* (13). The marked variations in the biotin content of 'normal' milk were also noted by Lawrence *et al.* and we agree that these variations are such as to mask any lactational trends after the first week. The mean values for the biotin content of milk from individual cows and from bulked milk from the N.I.R.D. herd (see Table 2) fall within the range of values published by other laboratories. It is beyond the scope of this paper to give a full account of the many values recorded in the literature for the B-vitamin content of milk. For them the reviews by Kon & Henry (16, 17), Gregory (18) and Porter (19) may be consulted.

The pantothenic acid curve was similar to the biotin curve, except that after the initial rise in the colostrum there was a gradual decrease from the 7th day throughout the lactation period. This observation supplements the findings of Lawrence *et al.* (13) who were able to follow pantothenic acid levels only up to the 10th week of lactation. The mean values for individual cows and the bulk milk sample fall within the ranges of 1·9–4·5 μg. pantothenic acid/ml. milk reported by other investigators.

The 'vitamin B_6' curve was similar in pattern to the pantothenic acid curve. The range of values for mature milk from individual cows (0·03–0·68 μg. 'vitamin B_6'/ml.) and the value of 0·31 μg. 'vitamin B_6'/ml. for bulk milk are lower than most published values which are in the range of 0·50–0·95 μg./ml. (18). In a more recent paper, however, Hodson (20) has revised his earlier value of 0·67 μg. 'vitamin B_6'/ml. milk. With an improved assay method, using *Neurospora* he found 0·33–0·35 μg. 'vitamin B_6'/ml., a value which agrees well with ours.

The steady decrease in the nicotinic-acid content of milk up to the 20th week of lactation and the subsequent rise towards the end of lactation were noted by Lawrence *et al.* (13). They did not observe the initial decrease and rise up to the 7th to 14th day shown in Fig. 2, and indeed it was not always apparent in the colostrum of individual cows. Lawrence and his co-workers found a mean nicotinic acid content of 0·61 μg./ml. of milk, a value in close agreement with ours. However, several workers have reported higher nicotinic acid levels of up to 1·1 μg./ml. (18).

As expected, riboflavin was high in colostrum and decreased rapidly during the first week to normal milk levels. Our values for milk of Shorthorn cows are sensibly the same as those obtained here by Houston *et al.* (2), but tend to be lower than those for bulk samples of milk collected in 1943–44 in Carmarthen, Banbury, Kent, Wiltshire, which according to Thompson (private communication), were predominantly from Shorthorn cows (Kon (21)). Since these bulk samples contained a small proportion of milk from other breeds richer in riboflavin, it is uncertain whether a relatively low riboflavin content is characteristic of milk of all Shorthorns or whether it is peculiar to the Shinfield herd. We have been unable to find any values for riboflavin in milk of Shorthorn cows elsewhere in Britain.

The vitamin B_{12} lactation curves were similar to those found by Anthony *et al.* (14) and Collins *et al.* (15) for Friesian cows. Both these groups of workers found higher levels of this vitamin in colostrum than in the milk. They also noted the wide range of values which we found, in milk from cows of the same breed and from individual cows on consecutive days.

Our results give some indication that the content of certain vitamins is affected by changes in the diet. Lawrence *et al.* (13) noted that during late winter and early spring the nicotinic-acid content gradually decreased until May and then rose until October. Hoeflake (22) and Stefaniak & Peterson (23) also noted a rise in nicotinic acid in market milk

during the summer. Stefaniak & Peterson found a seasonal variation in the biotin content of milk. But since the milk from cows fed on grass silage contained more biotin than the milk from cows fed on maize silage or pasture, they attributed the seasonal variation to changes in feeding which, they suggested, caused a change in rumen bacteria.

Although we were not able to detect any seasonal changes in riboflavin, Houston *et al.* (2) at this Institute noted an increase in the riboflavin content of Shorthorn milk when the cows went out to pasture. The cows used by Houston *et al.* in 1940 were kept on a winter ration of hay, mangolds, maize and concentrates, whereas the cows used in our later experiments were kept on a ration of hay, silage and concentrates. This change-over from mangolds to silage could account for our different results, since Theophilus & Stamberg (24) observed that the riboflavin content of milk increased when cows were put on to a feed of silage instead of a dry ration, and Johnson, Maynard & Loosli (25) found that the riboflavin content of milk was only influenced to a slight extent by the diet if the winter ration consisted of good-quality hay, grass silage and a grain mixture.

The effect of the diet on the vitamin content of milk has also been studied by Collins *et al.* (15). They suggest that the large variation in the vitamin B_{12} content of cow's milk may be due to the influence of dietary cobalt on the rumen synthesis of vitamin B_{12}. The giving of traces of Co, Cu, Fe, I and Mn 1 month before parturition increased the vitamin B_{12} level in goat's colostrum and milk during the 1st week of lactation (26). However, Hartman & Dryden (27) comparing the milk from cows, stall-fed in winter and on pasture in the summer, with and without supplementary feeding of trace minerals, found no effect of trace metals on the vitamin B_{12} content of the milk either in summer or winter, nor any difference between stall-fed and grazing animals. Similarly, Shrimpton & Duckworth (28) found no effect of supplementary cobalt on the vitamin B_{12} content of milk of ewes on lowland or hill grazing.

In Finland, Nurmikko & Virtanen (29) found no significant difference between the vitamin B_{12} content of milk from cows on pasture or on an indoor feed of hay and concentrates. However, when stall-fed animals were receiving plenty of AIV-silage (prepared from a mixture of clover and grass cut at an early stage of growth), the vitamin B_{12} content of the milk was significantly higher than when the cows were on pasture. Our own results also showed a fall in the vitamin B_{12} level in the milk when the cows went from indoor feeding on hay, concentrates and silage to pasture. But we were unable to confirm the statement of Nurmikko & Virtanen (29) that the vitamin B_{12} concentration in the milk increased as the milk production decreased. In fact, we found that the total vitamin B_{12} secreted per day was at its highest during the 1st month of lactation when both the milk yield and the vitamin B_{12} content/ml. were highest. Towards the end of lactation, as the milk yield decreased, there was no concomitant increase in the vitamin B_{12} concentration.

SUMMARY

1. Microbiological methods were used to measure biotin, nicotinic acid, pantothenic acid, riboflavin, 'vitamin B_6' and vitamin B_{12} in milk from Guernsey, Friesian and Shorthorn cows.

2. A study was made of day-to-day variations and breed differences in the B-vitamin content of such milk. Fluctuations in each vitamin were observed from one day to the next in milk from individual cows, these variations being especially marked with biotin

and vitamin B_{12}. In addition, the variations in the mean vitamin levels of milk from cows of the same breed were generally greater than any breed difference.

3. Samples of milk from ten Friesian and ten Shorthorn cows were taken at intervals throughout one complete lactation and analysed for each of the vitamins mentioned above. The results of this study of lactational trends are presented in detail.

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736. PHYSICAL CHANGES IN MILK CAUSED BY THE ACTION OF RENNET

III. EFFECTS OF SEPARATION, HOMOGENIZATION AND PASTEURIZATION AND OF VARYING CALCIUM CONTENT ON THE RIGIDITY MODULI AND VISCOSITIES OF CURD

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(With 4 Figures)

INTRODUCTION

In the first paper of this series (Scott Blair & Burnett (1) referred to as I), a method was described for measuring the increasing rigidity of renneted milk. It had already been shown (Scott Blair & Burnett (2)) that a cylinder of setting curd, once there is appreciable rigidity, behaves as a 'Burgers Body' when it is subjected to a series of small stresses, applied in alternate directions.

This means that the application of a load produces an immediate elastic displacement (modulus G_1) followed by a slow elastic displacement (modulus G_2) the elastic elements being partly damped by a viscous element (viscosity η_2). On removing the load, the reverse process occurs, i.e. there is an immediate recovery, followed by a slow recovery which is logarithmically related to time, from which relation the retardation times ($t_{ret.} = \eta_2/G_2$) can be calculated. There is also a small non-recoverable displacement corresponding to a 'creep-viscosity' (η_1). It was at first thought that measurements of η_1 were unreliable but they are now obtained with, probably, rather greater accuracy than are the values of η_2. The ratio $\eta_1/G_1 = t_{rel.}$ is defined as the relaxation time. It has been suggested (Scott Blair (3)) that the ratio $N = t_{rel.}/t_{ret.}$ may prove to be a good indication of 'springiness' and this has been called the 'springiness number'. The importance of this ratio was apparently first appreciated by Irmay (4), but his interest lay rather in its thermodynamic aspects and he did not associate it with 'springiness'.

It is well known that many factors, including calcium content, heat treatment, fat content and globule size-distribution affect the setting of curd by rennet. The purpose of the present investigation is to study quantitatively the effects of variations in such factors as these on the individual rheological constants of the curd.

In I it was recorded that, if the column of milk in the U-tube is not quite level at the moment when setting starts, a systematic difference ensues in the values of the moduli as determined in the two directions. This is due to the setting up of internal stresses in the gel. It has since been found that a frequent cause of this undesirable asymmetry is that the first traces of rigidity appear during the particular minute while the stress is being applied. It is advisable, therefore, not to make any measurements until about 2 min. after a small duplicate sample of normal milk has shown visible signs of setting. For slow-setting milks a correspondingly longer interval is advisible. As explained in I, the instrument is not, in any case, suited to measure shear moduli of less than about

10 dynes/cm.^{2} and quite different apparatus for studying the earliest stages of setting is in process of construction. The application to renneted milk of an instrument called the 'H.L. Torsiometer', designed for studying the setting of rubber latex, has already been reported (Scott Blair, Burnett & Lilley (5)). A detailed description of the Torsiometer is to be found in the original paper. Suffice it here to say that it measures the torque on a torsion wire from which a metal bob is hung in the setting curd, the torsion head being turned slowly in alternate directions. This instrument was used, for comparison with the U-tube gelometer, in some of the experiments here discussed.

It was stated in I that curd set in a U-tube generally shows appreciable syneresis only when subjected to stresses. In an ordinary glass U-tube, however, the stressing needed to make the measurements causes local detachments from the walls which invalidate measurements inconveniently soon after setting starts. In all later experiments, glass tubes etched with hydrofluoric acid have been used, and the renneted milk was inserted by means of a narrow filling-tube into the middle of the U-tube. Under such conditions, there is only a little syneresis before adequate data can be obtained, and the detachments occur almost entirely along the upper edge of the short curved part of the U-tube (see I).

Except right at the start of the setting, all curds containing adequate calcium and not showing visible syneresis, gave remarkably straight lines when G (the over-all modulus) or any of the individual Burgers parameters (G_1, G_2, η_1, η_2) was plotted against logarithmic time ($\log t$). This plot does not lend itself to direct interpretation in molecular terms but is convenient, first, as a check that syneresis is not introducing appreciable errors; and secondly, in giving two convenient constants, an intercept on the $\log t$ axis and a slope, by which the changing values of any parameter may be expressed. For the latter, it is convenient to write $t\dot{G}$ (etc.)* to represent the (constant) increase in G (etc.) when the time is doubled.

Moreover, these $\log t$ curves can be fairly safely extrapolated to a time, say, twice as long as the duration of the experiment and an approximate 'final' value of G (etc.) can be obtained. The parameters do, of course, increase still further with time but the values of G_∞ (etc.), obtained in this way, may be taken to approximate to those representing the complete setting of the casein. It is found that plots of $\log [(G_\infty - G)/G_\infty]$ (etc.) are reasonably linear with t, suggesting that, in the later stages of setting, the rate of increase of each parameter is proportional to the amount of still unset casein. In the earlier stages, it is probably also proportional to the amount of set casein. It should be noted that these two equations are incompatible, in the sense that both cannot be strictly true for all values of t; nevertheless, if a set of theoretical straight lines is taken from one plot and the figures are transferred to the other, the sigmoid curves so obtained are found to be remarkably linear over much of their length; certainly up to well over half the limiting values. It is perhaps somewhat surprising (and disappointing) that the $\log t$ plot is decidedly the better of the two, but this may be partly due to the inadequacy of the method available for determining the limiting values for the other plot.

Experimental

The present paper describes two experiments. In the first, samples of liquid milk taken from cows in the same herd at about fortnightly intervals, were tested raw, separated,

* $t\dot{G}$ (etc.) is written for '$t\dot{G}$, $t\dot{G}_1$, $t\dot{G}_2$, $t\dot{\eta}_1$ and $t\dot{\eta}_2$'. It should be remembered, however, that when these parameters are described below as 'rates of setting', they are really 'logarithmic rates', i.e. on a logarithmic time scale.

homogenized and heat-treated. In the second experiment, the effects of varying the calcium content of a reconstituted fat-free dried milk powder on the Burgers parameters, were studied.

Experiment 1

The three replicates of this experiment were made as alike as possible; except that, whereas on the first two occasions, the milk was pasteurized at 160° F., which changed its setting properties comparatively little, on the third occasion, it was deliberately overheated (180° F.). In the former case an A.P.V. plate-type heat exchanger was used; in the

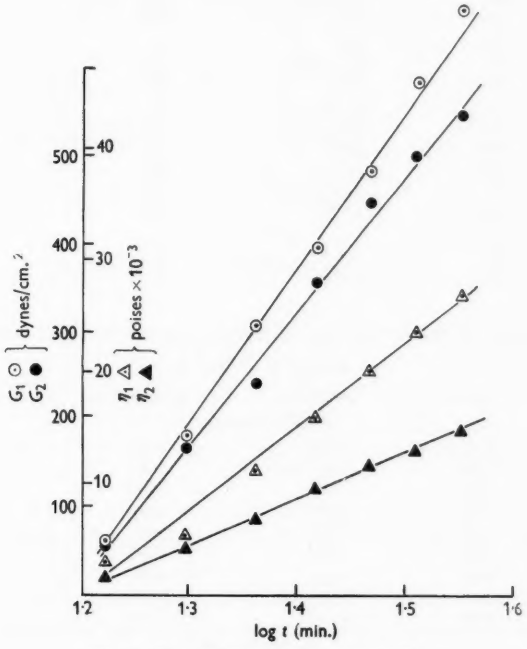

Fig. 1. Burgers parameters for raw milk.

latter, the milk was flash-pasteurized in a bucket. Separation was effected at 100° F. in a Lister separator. Homogenization, on the last two occasions, was done at 160° F. at 210 kg./cm.^{2} in a Rannie Homogenizer; and on the first occasion, with rather less precision, by putting the milk five times through a hand homogenizer at about 80° F. One of the twelve sets of curves is shown, by way of illustration, in Fig. 1. The accumulated data from all of the curves are given in tabular form.

The intercepts on the log time axis of each of the parameters for the three experiments are given in Table 1.

It should be noted that, since the lowest parts of the setting curves are not linear, the intercepts do not correspond exactly with the times when the curds first show signs of setting, though they are very closely related to these times, being slightly greater throughout. Since their measurement depends on the course of the whole of the later

part of the setting curve, they give a better indication of the behaviour of the milk than would the precise times at which rigidity could first be observed with the apparatus.

The corresponding rates of increase are shown in Table 2. The average values of N and of λ (the proportion of elastic recovery which is not immediate) are also included.

Table 1. *Intercepts (I) for parameters (min.)*

	Raw	Separated	Homogenized	Pasteurized
G	16·5 18 19·5	18 21 } +10 23	21·5 21 } +17 21	17·5 19·5 } +27 31·5
G_1	16 18 19·5	18 22 } +18 23	20 20·5 } +14 20·5	18 19·5 } +30 32
G_2	15·5 17 19·5	18 21 } +20 23	20 20·5 } +17 20·5	17 19·5 } +32 32
η_1	17 19·5 19·5	17·5 20·5 } +17 22·5	21 25 } +19 21	19 21 } +27 31·5
η_2	19·5 17 19·5	17·5 21·5 } +11 23·5	20 20 } +9 21	18 21 } +25 31
C	— 18 18	— 22·5 25 (?) } +32 (?)	— 21·5 20·5 } +17	— 19·5 30 (?) } +38 (?)

Table 2. *Rates of increase of Burgers parameters and average values of N and λ*

	Raw	Separated	Homogenized	Pasteurized
		$G \times 10^{-2}$ dynes/cm.		
$t\dot{G}$	2·52 2·36 2·44	2·82 2·82 } +6 2·79	4·47 4·06 } +23 3·30	2·27 1·75 } −14 1·65
$t\dot{G}_1$	5·25 5·25 5·25	5·85 6·22 } +3 5·52	8·20 5·87 } +6 6·21	5·52 5·08 } −7 3·04
$t\dot{G}_2$	4·48 4·48 4·48	5·22 5·22 } +3 4·27	8·20 5·88 } +12 7·10	3·58 3·23 } −11 3·05
		$\eta \times 10^{-4}$ poises		
$t\dot{\eta}_1$	3·14 3·29 2·72	3·30 3·15 } +2 3·05	5·23 5·53 } +23 6·22	2·82 2·27 } −11 2·18
$t\dot{\eta}_2$	1·83 1·72 1·59	1·78 1·90 } 0 1·65	2·64 2·27 } +24 2·93	1·19 1·33 } −27 0·98
		Arbitrary units		
$t\dot{C}$	— 63 64	— 67 65 } +6	— 63 67 } +2	— 58 44 (?) } −20
		Average values		
N	1·59 1·62 1·43	1·64 1·49 1·52	1·78 1·86 2·35	1·54 1·26 1·95
λ	0·50 0·54 0·54	0·53 0·54 0·58	0·51 0·49 0·46	0·54 0·56 0·52

Note. No great accuracy is claimed for the figures in the second place after the decimal point, where these are given.

In both tables, the single figures following each set of three experimental values give the average increase (as a percentage) of the parameter as a result of the treatment, i.e. referred to raw milk.

The following conclusions may be drawn:

Under the conditions of the experiment, all three treatments consistently increased the intercepts (virtually 'setting-times') on all parameters. Over-pasteurization had the greatest effect. The effects of homogenization and of separation were about equally marked. These last two treatments increased the rate of setting once it had started (though with separation, the effect is small), whereas heat treatment, especially when

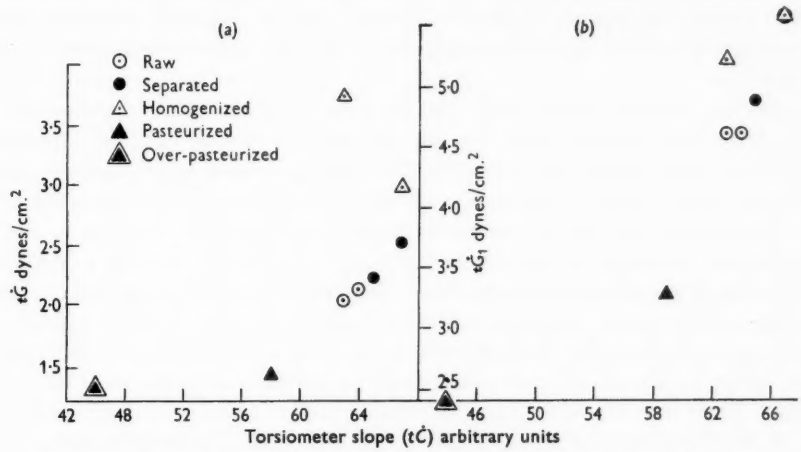

Fig. 2. Comparison of torsiometer slope ($t\dot{C}$) with over-all ($t\dot{G}$) and immediate ($t\dot{G}_1$) moduli.

excessive, reduced the rate of setting. Homogenization and also separation tended to alter the balance between immediate and slow elastic recovery. In the tables, C is the over-all consistency of the curd (in arbitrary units) as measured by the Torsiometer. This followed, reasonably well, the over-all modulus G, except in the case of homogenized milk, when it followed better the instantaneous part of the modulus G_1 (shown in Fig. 2).

Whereas the homogenization tended to decrease λ, the proportion of elasticity which is delayed, separation tended to increase this proportion. Pasteurization alone had little effect. It should be remembered, however, that the homogenization included heat treatment and, to a lesser extent, separation also did so.

Before closing this brief discussion on the data given in Tables 1 and 2, one further point should be stressed. It is, of course, already well known that pasteurization, especially if excessive, tends to retard the setting of milk by rennet and considerable work has been reported on the effects of homogenization and separation. The novelty in the present experiments lies, however, in that they give a much more detailed picture of the setting process following each treatment, so that the changes in each separate rheological parameter can be independently followed.

It is, perhaps, disappointing that N does not show more regularity. But it must be remembered that N is a ratio of two times, characteristic of the curd, each of which is defined as a ratio of a viscosity to a rigidity modulus. N is thus so far removed from the

original experimental data as to be subject to rather large errors, especially since (except perhaps in the case of homogenized milk) neither $t_{rel.}$ nor $t_{ret.}$ changed very much during the course of setting. With the limited data so far available, all that can be said is that homogenization may tend to increase the 'springiness number'.

It will be seen from Table 2 that $t\dot{G}_1$ and $t\dot{G}_2$ are about twice as large as $t\dot{G}$. In fact the instantaneous and delayed components of the modulus are about twice as large as the over-all modulus. This paradox, suggesting that the parts are greater than the whole, reflects the inadequacy of mechanical models. Although we can consider the stresses and deformations on the individual components of a model, rigidity moduli are calculated from *strains*, i.e. deformations relative to original lengths and we cannot measure the 'original lengths' of parts of a model. The paradox does not appear if, instead of moduli, we use their reciprocals (called 'compliances'), but this leads to difficulties in other directions.

Though not included in the tables, another very significant effect was observed. As stated above, even with raw milk, syneresis caused little trouble until the experiments had gone on for as long as was necessary. But homogenization and, to a lesser degree, separation, reduced greatly the amount of syneresis visible after the completion of the test. Over-heating, but not normal pasteurization, had much the same effect. Further experiments on syneresis are in progress and will be described in detail in a later paper. But it is already clear that the presence of fat globules, and especially large ones, increases the danger of detachment of the curd from the walls of the glass U-tube, particularly if these are smooth. Any point of detachment forms a centre for the accumulation of whey, i.e. for syneresis. The fat globules doubtless act by reducing the adhesion of the curd to the glass. There is also clear evidence that these points of detachment are formed in the parts of the wall of the tube where fat globules tend to collect, for hydrodynamic reasons. This does not, of course, imply that no syneresis is possible in fat-free milk, though it is normally much less marked than in full milk.

Experiment 2

In Fig. 2 of I, some preliminary curves are shown to illustrate the effect of adding increasing quantities of calcium chloride to a reconstituted fat-free dried milk. The values of G were later plotted against $\log t$ and found to give a series of remarkably parallel straight lines, except that the curves for samples containing but little added calcium only became linear after a considerable time. The complete set of these curves is shown in Fig. 3.

It was appreciated that the addition of increasing quantities of $CaCl_2$ would progressively lower the pH of the milk and it was decided to repeat the experiment adjusting the amount of added lactic acid so as to bring all the samples, before adding the rennet, to about the same pH (6·30–6·35), whatever the $CaCl_2$ content might be. Moreover, in the earlier experiment, G alone was measured: in the present experiment, all the Burgers parameters were determined.

Although the quantities of calcium added appear to vary over quite a wide range, it must be remembered that by no means all the calcium in the original milk had been rendered unavailable by the drying. A determination on a similar (but not identical) sample of dried milk showed that 100 ml. of milk gave 36 mg. of ultrafiltrable calcium. Nor is the effect of the additions of $CaCl_2$ on the pH very large. Comparing two samples of

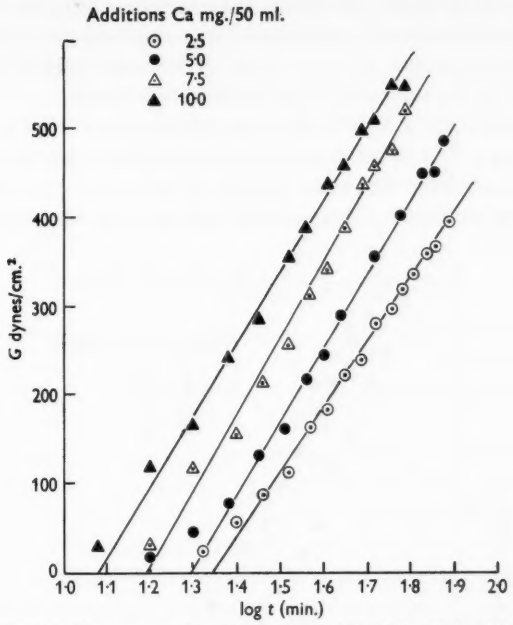

Fig. 3. Log time curves for setting of dried milk with added calcium.

Table 3. *Parameters for standard dried milk with varying additions of* $CaCl_2$, *all at about* pH = 6·3

		Ca mg./50 ml.							
		0	1·25	2·50	2·50	5·0	5·0	7·5	10·0
$\times 10^{-2}$	$t\dot{G}$	1·36	1·52	1·72	1·78	1·78	1·78	1·80	1·84
	$t\dot{G}_1$	2·79	3·42	3·42	3·80	3·62	3·55	3·43	3·73
	$t\dot{G}_2$	2·35	2·80	3·24	3·56	3·30	3·55	3·17	3·73
$\times 10^{-4}$	$t\dot{\eta}_1$	1·71	2·39	2·29	2·67 (?)	2·67	2·80	3·05	2·72
	$t\dot{\eta}_2$	0·89	0·94	1·14	1·14	1·14	1·27	1·21	1·27
min.	$\bar{T}$	19·5	17·5	14	15	15	15	12	12
sec.	$\bar{t}_{rel.}$	57·5	69	65	74	71·5	76	76	67
sec.	$\bar{t}_{ret.}$	37	32·5	31	32·5	34	34	33·5	33·5
	$\bar{N}$	1·60	2·10	2·07	2·28	2·11	2·11	2·27	2·10
	$\bar{\lambda}$	0·55	0·53	0·51	0·52	0·53	0·50	0·52	0·50
min.	t_0	19	14	12	11·5	12·5	11·5	11	10

50 ml., each containing 1 ml. 2% lactic acid, the first with no added calcium and the second with the maximum amount of 10 mg., it was found that the pH fell from 6·38 to 6·13. Moreover, it is not easy to reproduce pH values on milk powders to which appropriate amounts of $CaCl_2$ and lactic acid have been added with any very great accuracy.

The experiment was repeated a number of times and the results are shown in Table 3, including duplicate runs on two of the concentrations. The times at which visible evidence of setting first appeared (t_0) are also included.

The four Burgers parameters and the over-all modulus all show the same trends. With little or no additional calcium the values are rather small and the largest additions give appreciable increases in them. In the middle region, however, quite marked differences in

calcium content have no significant effect. The differences between samples having 2·5 and 5·0 mg. added calcium should be compared with the duplicate values at each of these concentrations. The constants, $t_{rel.}$, $t_{ret.}$, λ and N are not significantly affected, except perhaps in the case of the sample with no addition of calcium.

This tabular formulation is derived from the straight part of the curves when G (etc.) is plotted against log t. It is of interest to see what happens when the values of G_∞ (etc.) are found, as described above, and a plot is made of log $[(G_\infty - G)/G_\infty]$ against t. Curves of this plot are shown in Fig. 4. Many similar curves have been obtained from other experiments.

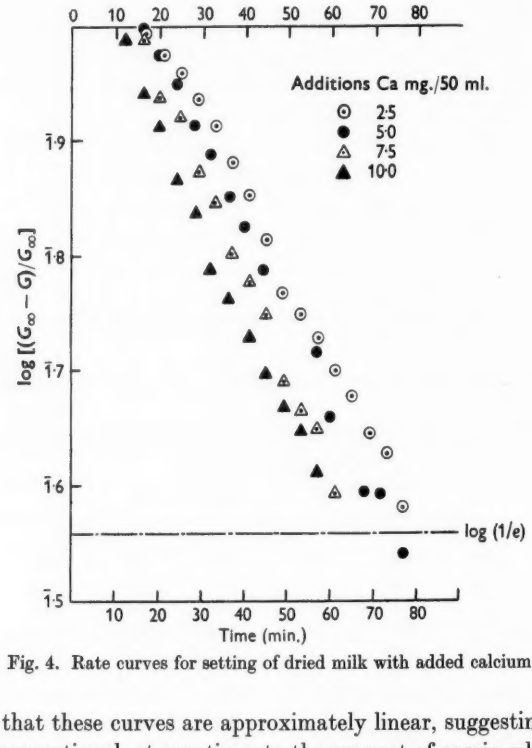

Fig. 4. Rate curves for setting of dried milk with added calcium.

It will be seen that these curves are approximately linear, suggesting that the curd is setting at a rate proportional, at any time, to the amount of casein which remains unset at that time; and that there is a general trend for the rate of setting to increase with increasing calcium content, if we consider the whole course of the setting process following the onset of rigidity.

DISCUSSION

It should be emphasized that the work described in this paper is essentially preliminary. Milk is a biological fluid. Very many samples must be tested before it is fair to say, for example, that 'homogenization increases the proportion of fast to slow elasticity of curd' or that 'addition of calcium chloride has little effect on the (log) rate of setting once the initial stages are complete'. Nevertheless, the data obtained from the limited number of experiments already carried out agree well enough among themselves to suggest that further work will probably substantiate such generalizations. In view of this situation,

however, it has seemed best to report the experiments here described in the past tense, e.g. 'the homogenized milk showed...' rather than to use the generalized present, 'homogenized milk shows...'.

A comparison of the shapes of the setting curves with those obtained by other authors using rather similar and also quite different methods is of much interest, but will be left for a later paper. In the meantime, much work remains to be done under carefully controlled conditions of treatment (pasteurization, homogenization, concentrations of calcium and hydrogen ions, etc.) to amplify these preliminary findings.

SUMMARY

1. Preliminary experiments show that homogenization, separation and pasteurization all tended to delay the onset of rigidity in renneted milk, though it must be borne in mind that the first two of these treatments were accompanied by some heating of the milk.

2. Once setting had started, homogenized and separated samples set more quickly, and pasteurized (especially over-heated) milk more slowly, than did raw milk.

3. Homogenization would seem to increase, and separation perhaps to decrease the proportion of elastic recovery of the gel which is immediate. The torsiometer, which gives an over-all measure of general consistency, agrees well with the U-tube gelometer but when the latter shows an unusual ratio of immediate to slow recovery, the torsiometer follows the former modulus.

4. Increasing quantities of $CaCl_2$ added to reconstituted dried fat-free milk progressively reduce the time needed for rigidity to make its appearance but do not greatly alter the final setting rates. All setting curves approximate to the law that, once rigidity is well established, rate of setting is proportional to the percentage of casein still unset. When the amount of added lactic acid is adjusted so as to prevent the addition of various quantities of $CaCl_2$ from altering the pH appreciably, no great difference is found in the setting curves.

5. The relaxation times, retardation times and their ratio, called 'the springiness number', are not greatly affected by the treatments to which the milk samples, either whole or dried, were subjected.

6. Appreciably syneresis usually occurs in the U-tube gelometer only when the curd has been strained, though very small strains are sufficient to produce it. The site of its occurrence depends on the hydrodynamic distribution of the larger fat globules. In fat-free or homogenized milk, such syneretic phenomena are either absent or very slight.

Our best thanks are due to Miss Helen R. Chapman for preparing many milk samples and to Dr R. Aschaffenburg who determined the pH values and the ultrafiltrable calcium. These and other colleagues at the N.I.R.D. have also given us most helpful advice. We also thank Dr P. White for help concerning the mathematical implications of the two methods used for plotting our data.

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737. THE HEAT COAGULATION OF MILK

II. VARIATIONS IN SENSITIVITY OF CASEIN TO CALCIUM IONS

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(With 2 Figures)

In a recent study of heat coagulation (1) attention was drawn to the unexpected effect of an intervening dialysis against raw milk on the heat stability of previously heated milk. Increased sensitivity to heat was found to result when previous heat treatment had been relatively mild, stabilization when it was more severe. These changes were accompanied by varying degrees of transfer of colloidal phosphate from the unheated to the heated milk as a result of dialysis.

Further examination has now shown that the phenomenon is due essentially to rather complex changes in the sensitivity of casein to calcium ions. Casein alone appears to be involved. Neither the formation of lactose-protein combinations nor the presence of serum proteins contributes to the effect, and the accompanying movements of colloidal phosphate also appear to be unrelated.

These variations in casein sensitivity develop fairly early in the heating process. While their recognition undoubtedly complicates the picture of heat coagulation already given, it can help to explain some anomalies not accounted for by the earlier simpler view.

In the account which follows these findings are described and discussed. Experimental methods and materials, unless otherwise stated, are those already used (1).

RESULTS

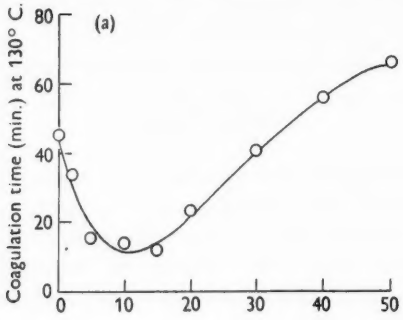
(1) *Heat sensitization of milk following previous heating and dialysis*

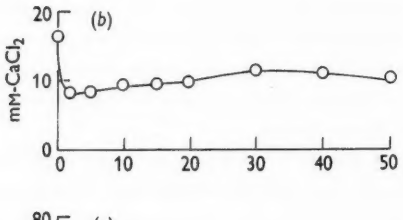
Fig. 1*a* shows the relationship of the coagulation times of skim-milk at 130° C. to the duration of its previous heating at 120° C. (dialysis against unheated milk intervened). Minimum stability at 130° C. is seen to follow some 10–15 min. previous heating at 120° C. Stability increases thereafter, re-attaining its initial value on about 30 min. heating at 120° C. and further improving as heating is prolonged. Similar stability patterns are shown by all milks, but minima are not always equally marked. Previous heating to 130° C. instead of 120° C. merely accelerates these developments.

(2) *Sensitivity to calcium of the casein from heated milks*

Sensitivity was measured by determining the minimum concentration of calcium chloride needed at 100° C. and pH 6·6, and in presence of 15 mM-NaCl, to effect incipient coagulation of a 2·5 % solution of the isolated caseinate in 3 min. The casein was isolated from the variously heated milk samples by isoelectric precipitation, then well washed, redissolved in sodium hydroxide solution at about pH 6·6 and brought to the necessary salt concentrations by dialysis.

Fig. 1*b* shows the relationship of the calcium sensitivity of the casein thus determined, to the duration of previous heating of the corresponding milk at 120° C. Casein stability is seen to develop an early minimum (in 2–5 min.), then rise to a maximum (in 30 min.) and thereafter gradually decline. Concomitantly the appearance of the caseinate solution before heating to 100° C. passes in turn from turbid to clear, and back again to turbid with





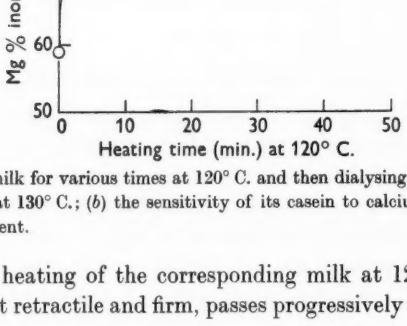


Fig. 1. Effect of preheating milk for various times at 120° C. and then dialysing it against unheated milk on: (*a*) its coagulation time at 130° C.; (*b*) the sensitivity of its casein to calcium chloride at 100° C.; (*c*) its inorganic phosphate content.

increase in the time of heating of the corresponding milk at 120° C.; and the type of coagulum formed, at first retractile and firm, passes progressively to gel-like under similar conditions.

Inspection of the curves for milk coagulability at 130° C. and for the corresponding caseinate coagulability at 100° C. reveals considerable similarity. It is difficult to escape the conclusion that heat-induced changes in casein sensitivity to calcium are basically responsible for the variations of coagulability shown by the heated milk of which the original serum condition has been restored by dialysis. Exact correspondence between the two curves is hardly to be expected in view of the different substrates and types of coagulation they illustrate.

(3) *Phosphate movements in heated and subsequently dialysed milk*

(*a*) The nature of these movements has been briefly referred to (1). Milk heated to 120° C. and neighbouring temperatures rapidly acquires the property of withdrawing phosphate from unheated milk when subsequently dialysed against the latter (Fig. 1*c*). Part of the transfer is, of course, due to replacement of the soluble phosphate removed from solution by heating, but an equal or greater part arises from an actual build-up of colloidal phosphate in the heated milk during dialysis. Estimations of total and dialysable inorganic phosphate in heated milk made before and after dialysis against unheated milk, establish this (unpublished result).

(*b*) Phosphate transfer may be strikingly demonstrated by reversing the usual dialysis conditions, e.g. by dialysing a relatively small quantity of unheated milk against heated milk. With increase in the severity of heating the susceptibility of the unheated milk to coagulation with rennet progressively diminishes and finally disappears altogether. Progressive withdrawal not only of its soluble calcium but also of its colloidal phosphate, a withdrawal not compensated for by increasing acidity, would appear to be responsible (cf. (2)). This phenomenon incidentally makes it impossible to determine calcium-ion concentrations of strongly heated milks using the dried-milk technique developed elsewhere (1).

(*c*) The quantity of colloidal phosphate which may be built-up by a heated milk, through dialysis against unheated, depends on the initial content of this colloid. A rough proportionality exists between the two. Thus milks of colloidal phosphate contents 80·9, 55·6 and 16·5 mg. % P_2O_5 heated for 50 min. at 120° C. increased in colloidal phosphate content by 21·2, 13·4 and 3·9 mg. % P_2O_5, respectively, on subsequent dialysis against unheated milk.

(Variation of colloidal phosphate content, up or down, as required, can be achieved conveniently by controlled alkalization or acidification of milk, respectively, followed by dialysis against considerable excess of the untreated milk. I am indebted to Mr C. McGann, of this department, for this technique and for preparation of the samples used here.)

(*d*) The normal sequence of heat-induced stability changes occurs in milk samples independently of their colloidal phosphate content (Fig. 2).

(*e*) A clue to the origin of the capacity of heated milk to build-up additional colloidal phosphate is provided in the work of Evenhuis & de Vries (3), who note the occurrence of somewhat analogous phosphate movements within strongly heated milk especially where finely divided solids are also present. These authors ascribe the movements of phosphate to conversion of the original amorphous labile colloidal phosphate, along with some of the soluble, to coarser, microcrystalline, less readily soluble material, possibly hydroxyapatite. The colloidal phosphate build-up dealt with in the present paper may perhaps arise from the further growth of such heat-developed phosphate crystal centres in the favourable serum conditions created by dialysis against unheated milk. A rough proportionality between original colloid phosphate content and that built-up in dialysis becomes understandable in such a case, as also does an observed failure of caseinate solutions heated in the complete absence of inorganic phosphate to build-up colloidal phosphate on subsequent dialysis against unheated milk.

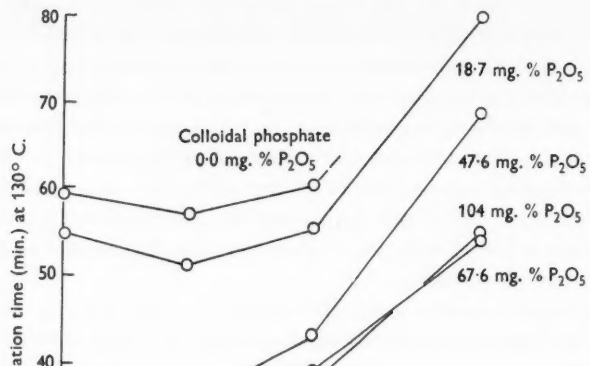


Fig. 2. Effect of colloidal phosphate content of milk on the coagulation time at 130° C. of samples preheated for various periods at 120° C. and dialysed against unheated milk.

(4) *Colloidal phosphate as a factor in heat coagulation*

Fig. 2 also illustrates the effect on heat coagulation of varying the initial colloidal phosphate content of a milk sample while keeping other factors constant. Progressive increase in colloidal phosphate content from 0 to 104 mg. % P_2O_5 is seen to bring about a corresponding progressive reduction of the coagulation time at 130° C. from 59 to 26 min.

In practice, the range of variation of colloidal phosphate will be much more restricted, perhaps only one-half as much as that used here, even with individual milks. The maximum variation in coagulation time arising from the operation of this factor taken in isolation should thus not exceed 50%.

(5) *Changes in soluble calcium and phosphate concentrations during heating*

In discussing earlier (1) the role of heat-developed acidity in the heat coagulation of milk, it was stated 'Developed acidity... is probably not an independent supplement of calcium-ion activity... but is also, and perhaps mainly, an indirect source of additional calcium-ions released from insoluble or deionising combinations. Investigation is needed on... these points however.'

Present investigations have not confirmed this opinion. Analyses of the dialysates from milks heated at 120° C. suggest that both soluble calcium and phosphate tend to fall rather than rise during heating despite the accompanying increase in acidity. Results of such analyses indicate that on three occasions losses of soluble calcium and phosphate of up to 14% had occurred just prior to coagulation, and on one occasion only had there been a slight rise in soluble calcium. Clearly, as noted by Evenhuis & De Vries (3) the phosphate which precipitates during heating shows little tendency to redissolve in acid.

How far, if at all, fall in soluble calcium is compensated for by an increase in the proportion of the element present as ions could not be determined by the methods employed. As already mentioned, attempts to estimate the calcium-ion concentration of heated milk by the renneting technique developed elsewhere (1) were unsuccessful.

(6) *Influence of developed acidity on heat coagulation*

Although developed acidity apparently fails to bring extra calcium into solution, it constitutes, through its hydrogen ions, an important supplementary factor in heat coagulation. Examples of its operation, additional to those reported (1) are:

(*a*) Minimum calcium-ion concentrations for the coagulation at 100° C. of casein isolated from heated milk can be reduced by about 2 m-mole/l. if the pH of the caseinate solution is lowered from 6·6 to 5·8.

(*b*) Periodic reneutralization to pH 6·6 of milk undergoing heating at 120° C. can delay almost indefinitely its coagulation.

(*c*) Prior small additions of substances which decompose on heating to yield acid and alkali, respectively, enable the coagulation time of milk to be reduced or increased at will. Thus with a milk which coagulated normally in 35 min. at 130° C. additions of 0·02, 0·05 and 0·10% gluconic lactone had the effect of reducing coagulation times to 28, 23 and 18 min., respectively; additions of 0·02 and 0·05% urea the effect of raising them to 62 and 104 min., respectively.

As the coagulation times in this series increased from 18 to 104 min. the hydrogen-ion concentrations of the corresponding milk samples at coagulation point also increased, viz. from pH 6·0 to 5·55. Higher acidities are presumably required for coagulation in the more slowly coagulating samples by way of compensation for lowered casein sensitivity and soluble calcium content.

(7) *Possible minor factors*

(*a*) *Serum proteins.* Presence of serum proteins has been shown to have little or no influence on the coagulation time of heated milk (1); it appears to have equally little on the course of development of heat-induced changes in the calcium sensitivity of casein. Very similar sensitivity patterns were obtained under the conditions used in § (1) with milks differing only in their content of serum proteins.

The synthetic milks compared here, 'albumen-poor' and 'albumen-normal', respectively, were prepared by dispersal of the bowl solids separated from chilled skim-milk by a Sharples supercentrifuge (25,000 r.p.m. for 10 min.) in milk dialysate, and neutralized, practically undiluted, isoelectric milk filtrate, respectively. Preparation of the latter was accomplished by controlled acidification with 10 N-HCl to pH 4·6, followed by filtration and subsequent neutralization of the filtrate by dialysis against several changes of the original milk. Both artificial milks were equilibrated by dialysis against the same skim-milk before being compared.

(*b*) *Lactose-protein combinations.* These combinations do not appear to contribute to the heat-induced sensitivity changes of casein: no significant differences in sensitivity to calcium could be detected in the casein isolated from identically heated milks, one composed of lactose-free milk +5% added lactose, the other of lactose-free milk +5% sucrose.

(*c*) *Caseinate particle size.* The milks prepared in §7*a* above from Sharples bowl solids

were found to coagulate in about two-thirds of the time taken by the corresponding original milk, presumably owing to the relatively coarse nature of their caseinate micelles. Conversely, Sharples centrifugate, containing about one-quarter of the original milk casein in the form of small particles, was found to coagulate (in 86 min. at 130° C.) about 5% more slowly than the original milk diluted to the equivalent casein content with dialysis-neutralized isoelectric milk filtrate.

Differences in degree of dispersion are perhaps not the only factors operating here since they are linked with differences in colloidal phosphate content (4,5). They may also be associated with slight compositional variation. Thus it was found that removal of colloidal phosphate from both the 'small-particle' and the normal, correspondingly diluted, milk hardly altered their relative coagulation times although, as usual, this treatment increased both to some degree.

It is noteworthy, and in keeping with earlier observations on the negligible part played by the serum proteins in the heat coagulation of milk, that even the Sharples centrifugate and correspondingly diluted milk coagulated very slowly despite a high 'albumin'/casein ratio of approximately unity.

DISCUSSION

The observations recorded here do not call for any essential revision of the general explanation of heat coagulation already given (1). They chiefly provide additional information on the significance of one factor, casein denaturation, which had previously been postulated but not examined in any detail, as well as some further evidence as to the operation of other factors. The present position, incorporating these newer results, appears to be as follows:

(*a*) Calcium-ion concentration, as modified in its operation by the presence of magnesium-ion and stabilizing anions, i.e. 'effective' calcium-ion concentration (1), probably remains the chief predetermining factor in heat coagulation. Its influence is reflected in the dominant role of the milk serum in heat coagulation. Thus replacement of the sera of milks of differing stability by a common serum has been shown to exercise a levelling influence on their stability (1); replacement of their various caseinate-phosphate complexes by a common complex, on the other hand, leaves the order of coagulability of the milks in great part unchanged (unpublished observation).

White & Davies (6) have been able to confirm the importance of calcium-ion concentration for heat coagulation in the case of relatively unstable milks, but not for those of medium or great stability. They remark: 'It may be that in milks which coagulate quickly the constituents likely to accelerate coagulation are sufficiently above a certain level to enable them to play their expected roles before major chemical changes brought about by prolonged heating made the initial composition of the milk no longer significant.' This is indeed highly probable. And perhaps such chemical changes may include that which underlies the complex and unexpected variations in casein sensitivity dealt with in the present paper, and discussed more fully below.

(*b*) The view that colloidal phosphate content is a second predetermining factor in heat coagulation appears to be confirmed, provided, of course, that conclusions drawn from milk in which the colloidal phosphate content has been artificially altered may be extended to normal milks. White & Davies (6) agree that 'the amount of colloidal phosphate attached to casein is probably an important controlling factor in the mechanism of

heat coagulation' but find, surprisingly, that in milk of relatively high colloidal phosphate/casein ratio, heat stability is correlated with calcium-ion concentration. Whatever its explanation, this result suggests that it is desirable to examine more closely the validity of the extension referred to above. This point is receiving attention.

(*c*) Acidity developed during heating still remains the most important supplementary factor in heat coagulation. Contrary to earlier expectation, however, it would appear to function mainly because it increases hydrogen-ion concentration. Only to a minor degree, if at all, is it an indirect source of calcium-ions. The calcium phosphate which is precipitated when milk is heated dissolves in fact only very slowly in weakly acid solutions (3).

(*d*) Casein denaturation, previously regarded as a factor of importance only in the later stages of heating, is now seen to play a vital role relatively early in the heating process. In milk, its effect is to create in the early stages of heating an increased sensitivity to calcium, which reaches a maximum after about 10–15 min. at 120° C., and is followed thereafter by a progressive decline. In casein, isolated from heated milk, these changes are reproduced, but with some slight differences. Maximum sensitivity to calcium and subsequent decline develop more rapidly, and the decline is in turn overtaken by a second, if slower, increase in sensitivity. These differences are not unduly great considering the different materials and coagulation conditions employed in the two cases.

The fall in the sensitivity of milk casein to calcium-ions which sets in after a comparatively short heating time at 120° C., together with the general tendency of soluble calcium to fall during heating, would probably lead, in the case of milks of moderate or low initial calcium-ion concentrations, to increasing stability as heating proceeds, were it not for the simultaneous increase in acidity. Under the varying conditions of balance of these opposing factors the tendency of such milk to coagulate may remain almost constant for considerable periods, or even fluctuate. Under these conditions it is not surprising that the correlation between coagulation time and calcium-ion concentration should be poor for milk of moderate stability.

Increased casein stability may also explain the high-temperature fore-warming treatments for improving the heat stability of evaporated milk studied by Webb & Bell (7). It is significant that, as noted by Webb, Bell, Deysher & Holm (8), the general heat stability relationships of such fore-warmed milks are not affected either by development of acidity in the milk or addition of stabilizing salts to the concentrated milk. They would thus appear to arise from changes in the colloidal constituents of milk, most probably from heat-induced changes in the sensitivity of casein to calcium similar to those discussed in the present paper.

(*e*) Neither serum protein nor lactose-protein combination appears to play any significant part in the heat coagulation of milk, or in the development of the changes observed in the calcium sensitivity of casein.

SUMMARY

1. The sensitivity to calcium of the caseinate-phosphate complex of milk heated at 120° C., increases to an early maximum and thereafter steadily declines, apparently in consequence of parallel changes in the sensitivity of its caseinate constituent.

2. A simultaneously developed capacity of the heated complex to bind additional colloidal phosphate appears to be unrelated to these changes in casein sensitivity.

3. Colloidal phosphate content is confirmed as a factor in the heat coagulation of milk.

4. Heat-developed acidity contributes to the heat coagulation of milk primarily by increasing hydrogen-ion concentration and only slightly, if at all, through release of calcium ions from insoluble combinations.

5. Neither serum proteins nor protein-lactose combinations play any significant part in the heat coagulation of milk.

6. The bearing of these results on the heat coagulation of milk in general and on the operation of certain high-temperature stabilization treatments is discussed.

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738. PHOSPHOLIPIDS IN NEW ZEALAND DAIRY PRODUCTS

III. EFFECT OF STORAGE ON THE PHOSPHOLIPIDS AND ON THE PARTITION OF PHOSPHORUS IN BUTTER

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The breakdown of organic phosphorus compounds during storage of butter at −10° C. was reported by Cusick in 1920 (1). He found that all butters, whether from pasteurized, unpasteurized, ripened or sweet cream, increased in soluble inorganic phosphorus content during storage. He found also that there was a more rapid decomposition of organic phosphorus compounds in salted than in unsalted butter.

Other workers, investigating the cause of 'fishy' flavour in stored butters, inferred from the presence of trimethylamine* that lecithin decomposed during storage (2). Unpublished results from the investigations of an earlier worker at this Institute also indicated that breakdown of the phospholipids might occur during storage of salted butter.

Investigations over many years have led to a fairly clear understanding of the various types of phosphorus compounds in milk. In the earlier work the proportions of soluble and insoluble phosphorus were estimated after filtration or dialysis of the milk (3). Later, the partition was determined after precipitating the proteins in the milk with picric or hydrochloric acids or, more recently, with trichloroacetic acid (3,4,5). The partition of phosphorus in butter, however, has received relatively little attention. Cusick (1) estimated the soluble and insoluble phosphorus contents of a number of butter samples after extracting them, first with a half-saturated sodium chloride solution, and then with 0·2% hydrochloric acid. His results are of considerable interest though hardly comparable with the results of later work in which trichloroacetic acid has been used as the protein precipitant. More recently, results on the distribution of phosphorus in skim-milk, buttermilk and butter serum from the same whole milk were reported by Nielsen (6).

SOURCE OF BUTTER SAMPLES

(1) *Institute experimental factory*

Unsalted butter and butters containing 1·6 and 2·5% of salt were made from portions of the same bulk cream at the Institute factory on five occasions over a period of 5 months. One lb. pats from each churning were stored at −10° C. for 8 months.

(2) *Commercial butter factory*

Twice monthly over a period of 10 months a sample (1–2 lb.) of freshly made butter, salted to 1·6%, was obtained from a commercial factory in the Manawatu district of the North Island. A small portion of the sample was withdrawn for analysis and the remainder of the sample was stored at −10° C. for 8 months.

* The theory that 'fishiness' in butter is caused by trimethylamine is now disproved (2).

EXPERIMENTAL

Preparation of serum from butter samples

Careful melting and centrifuging of butter at a temperature of under 50° C. ensures that only a trace of phosphorus is found in the butterfat (7). Thus only the serum is required for a quantitative evaluation of the phosphorus fractions, and it was prepared from the butter (70–80 g.) by the method described in Part I of this series (7). The quantity of butter sample was reduced to 11 g. and the method of preparation of the serum was modified when results only for phospholipid P were required (see Part I (7)).

METHODS OF ANALYSIS OF BUTTER SERUM

(1) *Separation of various phosphorus fractions and estimation of phosphorus*

The methods described by Graham & Kay for milk (4) were adopted, with suitable modifications, for the analysis of butter serum. The methods in detail were as follows:

(*a*) *Total* P. Butter serum (1 g.) was diluted with water to a final volume of 150 ml. and 1 ml. of the diluted serum was transferred to a $6 \times \frac{3}{4}$ in. Pyrex test-tube for digestion and estimation of P.

(*b*) *Fat-soluble* P. To a weighed quantity of serum, approximately 1 g. in a small conical flask, 4 ml. of water and 5 ml. of 25% trichloroacetic acid previously cooled to 4° C. were added. After 5 min. the liquid was filtered through a 9 cm. No. 42 Whatman paper. A portion (1·5 ml.) of the filtrate was diluted to 10 ml. with water and 1 ml. of the diluted filtrate was taken for digestion and estimation of P.

(*c*) *Inorganic* P. One ml. of the undiluted filtrate from (*b*) was transferred to a test-tube together with 4·5 ml. of water and 0·5 ml. of 25% trichloroacetic acid, and 0·5 ml. of 1% ammonium molybdate solution and 0·3 ml. of 1:2:4-aminonaphtholsulphonic acid reagent (prepared according to King (8)) were added. After 30 min. the intensity of blue colour was measured in a Beckman spectrophotometer at 830 mμ.

(*d*) *Phospholipid* P. The butter serum was extracted with ether and light petroleum according to the modified Röse–Gottlieb procedure described in Part I (7). One ml. of the mixed extract was taken for digestion and estimation of P.

(*e*) *Digestion of samples and estimation of* P. Samples from (*a*), (*b*) and (*d*) (above) were digested, and the total P in the digest was estimated by the method described for butter-serum extracts in Part I (7).

(2) *Estimation of lecithin, cephalin and sphingomyelin*

The quantities of each individual phospholipid in the mixed ether light-petroleum extract from the serum (see 1(*d*) above) were estimated by the procedure already described in Part I (7).

Calculation of results

Percentages of total P and of acid-soluble, inorganic and phospholipid P in the butter serum were calculated from the analytical results. Results for other phosphorus fractions were calculated as follows:

Acid-soluble organic P = acid-soluble P − inorganic P;
Acid-insoluble P = total P − acid-soluble P;
Casein P = acid-insoluble P − phospholipid P.

The calculation from the analytical results of the contents of total phospholipids and of lecithin, cephalin and sphingomyelin in butter was based on the factors used in Part I (7).

RESULTS

(1) *Partition of phosphorus in fresh butter serum*

Results for the total P content and for the P content of the various fractions after partition of the sera from 5 samples of fresh unsalted butter made at the Institute factory are shown in Table 1. Average results for the five butter sera and also some typical results for cow's milk are shown in Table 1. Graham & Kay's values (4) are the averages for the evening milks of five Jersey cows. Chanda & Owen's (9) values are the average results for samples taken from two to five cows at intervals from the third to the forty-second week of lactation.

Table 1. *Results for total phosphorus and for the partition of phosphorus in the sera from five samples of fresh unsalted butter; and comparison of the average results with some typical results for milk*

		Phosphorus content (mg./100 g.)						
	Sample	Total P	Acid-soluble P	Inorganic P	Acid-soluble organic P	Acid-insoluble P	Phospho-lipid P	Casein P
Butter serum	churned 26. ix. 52	86·0	34·1	29·0	5·1	51·9	33·2	18·7
	churned 19. xii. 52	91·7	35·9	32·1	3·8	55·8	36·5	19·3
	churned 13. i. 53	68·5	27·0	23·5	3·5	41·5	27·8	13·7
	churned 23. i. 53	85·4	32·8	29·9	2·9	52·6	36·3	16·3
	churned 30. i. 53	86·2	32·0	29·6	2·4	54·2	34·2	20·0
Average (butter sera)		83·6	32·4	28·8	3·5	51·2	33·6	17·6
Milk (Graham & Kay(4))		111·9	84·1	71·3	12·8	27·8	—	—
Milk (Chanda & Owen(9))		116·0	84·6	74·4	10·2	31·3	—	—

The acid-insoluble P content of butter serum was greater than that of milk, but the contents of acid-soluble and inorganic P were less than half the corresponding values for milk (see Table 1). Calculated for a phospholipid-free serum, however, the average proportions of acid-soluble (64·7%) and inorganic P (57·4%) in the total P of the serum were fairly close to those (75·2 and 63·7%, respectively) for milk.

(2) *Effect of storage of butter on the partition of phosphorus and on the phospholipids in butter serum*

Results for acid-soluble and inorganic P in the salted and unsalted butters made at the Institute were the same before and after storage. Average results for the phospholipid P contents of the sera from the stored butters also agreed very closely with those of the fresh samples (see Table 2).

Table 2. *Effect of storage on the average phospholipid* P *contents of the sera from unsalted and salted butters*

	Salt content	Phospholipid P (mg./100 g.)	
Sera from	(%)	Fresh	Stored
Unsalted butter	0	33·6	33·6
Salted butter	1·6	31·8	31·7
Salted butter	2·5	29·8	30·1

Average results for total phospholipid contents of nineteen samples of commercial butter before and after storage, and average results for contents of lecithin, cephalin and sphingomyelin in five of these butters before and after storage, are shown in Table 3. There was little or no change in the contents of total and individual phospholipids during storage.

Table 3. *Effect of storage on the average total phospholipid content and on the average lecithin, cephalin and sphingomyelin contents of salted butters*

No. of samples	Phospholipid estimated	Phospholipid content (%)	
		Fresh	After storage for 8 months
19	Total phospholipids	0·135	0·134
5	Total phospholipids	0·118	0·116
	Lecithin	0·033	0·034
	Cephalin	0·041	0·041
	Sphingomyelin	0·044	0·041

DISCUSSION

(1) *Partition of phosphorus in fresh butter serum*

The total P content of butter serum is dependent upon several factors which include (*a*) the total P content of the original milk, (*b*) the extent of dilution of the milk and of the cream with water, (*c*) the extent of the loss of phospholipids from the fat globule adsorption layer to the buttermilk during churning, and (*d*) the extent of washing and draining of the butter granules.

It will be seen from the results in Table 1, however, that in four of the butters analysed there was little difference in total P contents and in the P contents of the various fractions. The lower results for the fifth sample (churned on 13 January 1953) did not appear to be caused by any difference in manufacturing conditions, but could have been due to a lower total P content of the cream from which the butter was made since this cream was drawn from a different locality.

Dilution of the cream and washing of the butter would partly account for the lower results for acid-soluble and inorganic P in butter serum compared with milk. Dilution with water, however, was probably not the only factor involved. Nielsen (6), for example, found that the inorganic P contents of butter sera from non-washed butters were only 50–60% of those in the corresponding skim-milks. He suggests that the lower values were due to less complete formation of calcium phosphate-caseinate complexes in butter serum than in skim-milk.

The higher results for acid-insoluble P contents of butter sera compared with the values for the milks sampled by Graham & Kay and by Chanda & Owen (see Table 1) are explained by the increase in phospholipid contents* due to (*c*) above. Since the average phospholipid P content of milk was found by the present author to be 1·5 mg./100 g. (10), the amount in butter serum was twenty times as great as that in milk.

* Average results for phospholipid P contents of these milks were 10·0 mg./100 g. (Graham & Kay (9)) and 13·2 mg./100 g., (Chanda & Owen (9)). These results appear far too high, however, in comparison with those obtained by the present author (10) and by other workers (11, 12). The alcohol-ether extraction of milk used by Graham & Kay (4) and by Chanda & Owen (5) has, in fact, been shown to extract considerable quantities of non-phospholipid P (13, 14).

(2) *Effect of storage*

Storage of salted or unsalted butter had no effect on the total or individual phospholipid P contents of the butter sera. This finding does not preclude the possibility of autoxidation of the highly unsaturated fatty acid fraction of the phospholipid molecule during storage—the compounds formed from this reaction may be the cause of 'fishiness' and other undesirable flavours in stored butters (15). In the present investigation, however, there was no evidence of the separation from phospholipids or other organic phosphorus compounds of the phosphate group in an acid-soluble form or as inorganic phosphate. Thus the author is unable to confirm the findings of earlier workers who reported the occurrence of decomposition of the phospholipids or other organic phosphorus compounds during storage of sweet cream butter.

SUMMARY

Average values for the total phosphorus content of sera from unsalted butter samples and for the phosphorus contents of the various fractions of the sera after partition with trichloroacetic acid were: total P, 83·6 mg./100 g.; acid-soluble P, 32·4 mg./100 g.; inorganic *P*, 28·8 mg./100 g.; acid-insoluble P, 51·2 mg./100 g.; and phospholipid P, 33·6 mg./100 g.

There was no evidence that decomposition of the phospholipids or other organic phosphorus compounds occurred in salted or unsalted sweet-cream butters during storage at $-10°$ C. for 8 months.

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637·333

739. THE EFFECT OF HEAT TREATMENT AND TEMPERATURE OF STORAGE ON THE YIELD, QUALITY AND CHEMICAL COMPOSITION OF KARISH CHEESE

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INTRODUCTION

Separated milk cheese is made in Egypt in greater quantities than any other variety. If not sold fresh it dries up quickly and in addition to losing weight it becomes tough, hard and of poor commercial quality. Thus from the economic point of view it is important that the cheese should not deteriorate in quality on storage.

Fahmi (1) was one of the first investigators to improve the quality and manufacture of this cheese. He recommended certain technological processes of manufacture which improved the quality of separated milk cheese (Karish cheese), and studied the effect of different salt concentrations on the chemical composition of cheese. Moreover, his work suggested new lines of investigation. He developed a new and simplified technique using the acid-rennet method which offers a good basis for speedy and satisfactory making of Karish cheese.

El-Sadek & Abd el Motteleb (2) found that heating milk to 170° C. for 15 min. before making into Karish cheese resulted in a higher yield of materially improved quality compared with that of cheese from raw separated milk.

The object of this work was to study the effect of heating the milk prior to its manufacture and also the effect of storage temperature upon the quality and yield of this type of cheese. Quality changes were investigated by chemical determinations of some of the constituents of the cheese and of the whey in which it was pickled.

EXPERIMENTAL

The procedure followed in earlier work (2) was adopted. Four experiments were carried out. In each experiment a given amount of separated milk, standardized to about 1·5% fat, was made into cheese. The fat content was standardized to ensure the same fat content in the resultant cheese. Prior to making the cheese the milk was salted to 7% and divided into two equal parts. One was left raw, while the other was heated to 170° C. for 15 min. This temperature was chosen because it is customary in Egypt to heat one-third of the milk to this temperature and then to add it to the other two-thirds to bring the whole to renneting temperature. Rennet was added in the proportion of 25 and 33 ml. per 100 lb. of the raw and pasteurized milk, respectively. The cheese was removed from the moulds after 4 days and draining whey was collected for use as a pickling solution for the cheese.

In each experiment 2000 g. of each of the raw and pasteurized milk cheese were divided into four equal portions. Each portion was packed in a tin with 900 ml. of its own whey, and the tins were then hermetically sealed by soldering. Of the eight tins in

each experiment, four were kept at room temperature (*ca.* 30° C.), while the other four were kept in cold storage at 4–6° C.

After 4 weeks' storage cheese from four tins representing raw and pasteurized milk and the two storage temperatures were weighed, graded by three persons, and analysed; the remaining four tins were examined similarly after 8 weeks' storage. Flavour was marked to a scale, with a maximum of 50, while body and texture were each marked to a scale with a maximum of 25. The averages of the marks awarded by the three persons were recorded.

Methods of sampling and analysis of cheese and whey

These were the same as in the previous work (2).

RESULTS AND DISCUSSION

Quality of cheese

The marks awarded for flavour, body, texture, and the total marks for raw milk and pasteurized milk cheese stored under the different time and temperature conditions are shown in Table 1. It may be seen from this Table that the cheese made from pasteurized milk gained higher marks than that made from raw milk. This was so whether the cheese

Table 1. *Grading scores of raw milk and pasteurized milk cheese after storage at room temperature and low temperature for* 1 *and* 2 *months*

	Flavour (50 marks maximum)		Body (25 marks maximum)		Texture (25 marks maximum)		Total mark	
	Room temp.	Low temp.	Room temp.	Low temp.	Room temp.	Low temp.	Room temp.	Low temp.
				(*a*) After 1 month's storage				
Cheese made from raw milk	22·5	40	11	21·5	16	16·5	49·5	78·25
Cheese made from pasteurized milk	27·5	45	14·25	23	21·5	24·0	63·25	92·0
				(*b*) After 2 months' storage				
Cheese made from raw milk	18·5	34·5	10	20·5	15	15·5	43·5	70
Cheese made from pasteurized milk	20·0	37·5	11·5	20·5	20·5	22·0	52·0	80

was stored at room temperature or at low temperature. The effect of pasteurization may be to produce a softer curd capable of holding more moisture which helps to improve both flavour and body of the cheese. Pasteurization also seems materially to improve the texture of the cheese.

The cheese held at low temperature gained higher marks than that held at room temperature, whether it was made from raw or pasteurized milk. The beneficial effect of storage at low temperature may be due to the suppression of acid-producing organisms and increased moisture retention by the cheese. These two factors, of low acidity and appropriate moisture content, help to confer a palatable flavour and good body. It seems also that this low temperature of storage also helps to suppress the harmful effect of organisms surviving pasteurization on the texture of the cheese.

The same conclusions were arrived at when cheese was stored for 2 months. Table 1 also shows that the effect of storage at low temperature is much more important than the

effect of pasteurization of milk prior to cheese-making, in improving the quality of the cheese after storage.

Yield of cheese

Table 2 shows the minimum, maximum and average decrease or increase percentage in the yield of cheese made from raw and pasteurized separated milk after storage at room and at low temperature for 1 and 2 months. The losses in weight of soft cheese when stored at room temperature are well known and have been reported by many workers. El Baroody (3) reported that Damietta cheese (a whole-milk soft variety) lost about 33·7–38·5% after 3 months' storage at room temperature. There is no doubt that the losses in weight observed in cheese stored at room temperature are due largely to the escape of moisture. The losses were more noticeable in pasteurized milk cheese than in raw milk cheese owing to the higher initial moisture content of the former. It may be noted, however, that with soft cheese kept in a pickling solution the losses in weight are also due to the escape of other water soluble constituents. This will be shown later from the chemical analyses of cheese and whey.

The increase in weight of the cheese held at low temperature during the first month may have been due to absorption of some of the pickling solution in which the cheese was kept in tins. That cheese is capable of absorbing outside moisture has been reported by other workers. Van Slyke (4) kept one cheese under a bell jar in which the air was completely saturated with moisture. He found that after 1 month it had not changed in weight, but after 15 months it had gained 2% in weight. Further, it may be noted that the increase in weight of the pasteurized milk cheese was greater than that of the raw milk cheese. This may be due to a softer curd resulting from pasteurization which is capable of absorbing more moisture than the ordinary raw milk curd.

After the second month of storage, however, there was a loss in weight of cheese, both raw and pasteurized milk, stored at the low temperature. This decrease resulted from the expulsion of moisture from the cheese during this period of storage, this being greater in the pasteurized than in the raw milk cheese. This is to be expected because the amount of water absorbed by the former was greater than that absorbed by the latter, and thus after this second period the loss tended to be greater with the pasteurized milk cheese. A decrease in weight following an increase during the first month has been reported by other workers. Hamed (5) studied the effect of storage at temperatures lower than 4° C. on the yield of Damietta cheese (made from full-cream milk), and found an increase in weight after 1 month, followed by a small gradual decrease during the following months, which was smaller than the previous increase. The relatively higher storage temperatures used in this study may have produced decreases which excluded those reported by Hamed. It would appear that the lower the temperature of storage the smaller is the decrease which follows the initial increase during the first month. The decreases in the yield of cheese after 2 months' low-temperature storage were 6·6 and 13·95% for raw and pasteurized milk cheese, respectively, and these decreases agree with the findings of El Baroody (3), who reported a loss of 14·3–15·4% after 3 months' storage in cold rooms.

Changes in chemical composition of cheese and whey during storage

(*a*) *Moisture content of cheese.* Table 2 also shows the minimum, maximum and average moisture content of the cheese during storage. With storage at room temperature the

moisture content decreased over the 2 months and particularly during the first month. With storage at the low temperature the moisture content increased during the first month, the increase being slightly greater in the raw milk cheese. This was followed by a decrease at the end of the second month which was greater in the pasteurized milk cheese. The changes in moisture content during low-temperature storage correlate with the increases and decreases in yield of cheese, and movement of water-soluble constituents may also play a part.

(*b*) *Fat content of cheese.* Table 2 also shows the minimum, maximum and average fat content in the dry matter of the cheese. The fat content of cheese held at room temperature increased, due no doubt to the continuous loss of moisture from cheese stored at this temperature. The fat content in the dry matter of both raw and pasteurized milk cheese held at low temperature first decreased and then increased. However, the decrease observed after the first month of storage is only apparent and is due to the increase in weight of the cheese which occurs as the result of absorption of pickling solution by the cheese during this period. The increase in the fat content observed after the second month is attributable to the decrease in weight of the cheese due to the expulsion of moisture during that period.

(*c*) *Sodium-chloride content of cheese.* Table 2 also shows the minimum, maximum and average sodium-chloride content in the dry matter of raw and pasteurized milk cheese. There was a decrease in the average sodium-chloride content in the dry matter of raw and pasteurized milk cheese stored at room temperature. This decrease would result from the decrease in the moisture content which occurred during storage at room temperature as the sodium chloride is held in solution.

Table 2. *Percentage changes in yield and composition of cheese made from raw and pasteurized milk and stored for* 1 *and for* 2 *months at room and at low temperature*

			Storage at room temperature				Storage at low temperature			
	Initial		1 month		2 months		1 month		2 months	
Property	R	P	R	P	R	P	R	P	R	P
					Yield					
Minimum decrease or increase	—	—	−25·6	−35·34	−31·8	−37·6	+ 8·2	+10·3	− 2·8	−10·4
Maximum decrease or increase	—	—	−35·8	−39·8	−40·0	−40·6	+14·2	+15·6	−10·2	−17·6
Average	—	—	−31·05	−37·3	−35·5	−39·5	+10·85	+14·05	− 6·6	−13·95
					Moisture content					
Minimum	68·45	71·04	61·60	63·98	60·27	62·30	69·96	72·68	65·12	67·98
Maximum	71·96	74·18	64·45	65·88	61·12	64·22	72·79	75·09	69·18	70·84
Average	70·12	72·84	62·64	64·67	60·75	63·34	71·85	74·26	67·91	69·77
					Fat content in the dry matter					
Minimum	18·75	17·97	21·35	20·86	21·45	21·60	18·18	17·05	18·26	18·31
Maximum	20·48	19·38	24·06	23·91	24·67	24·39	19·57	18·66	21·02	20·85
Average	19·74	18·84	22·71	21·93	23·89	22·59	18·84	17·88	19·79	19·69
					Sodium-chloride content in the dry matter					
Minimum	15·05	16·42	11·36	12·52	10·82	11·97	16·11	18·37	13·04	14·74
Maximum	17·93	20·79	12·57	13·65	11·70	13·35	20·23	21·75	16·09	17·42
Average	16·38	18·93	11·95	13·26	11·21	12·57	17·97	20·47	15·11	16·29
					Total nitrogen content in the dry matter					
Minimum	5·43	5·62	5·12	5·33	5·08	5·25	5·41	5·59	5·35	5·50
Maximum	6·81	6·93	6·32	6·35	6·22	6·30	6·80	6·90	6·70	6·77
Average	6·04	6·17	5·69	5·84	5·65	5·75	6·01	6·15	5·95	6·04

R = Cheese made from raw milk. P = Cheese made from pasteurized milk.

With cheese held at low temperature, the average sodium-chloride content in the dry matter of both raw and pasteurized milk cheese first increased and then decreased. These changes followed the changes in the moisture content of the cheese stored at low temperature.

(*d*) *Total nitrogen content of cheese.* Table 2 also shows the minimum, maximum and average total nitrogen in the dry matter of raw and pasteurized milk cheese. The total nitrogen of raw and pasteurized milk cheese calculated on the dry matter showed a decrease when held at both room and low temperatures. This decrease in the total nitrogen content of the cheese may be due to the formation of water-soluble protein derivatives during ripening and their subsequent loss in the whey draining from the cheese. This is supported by the fact that the decrease in the total nitrogen continued all through the storage period, and also by the fact that the decrease was more pronounced when cheese was held at room temperature when ripening would be more rapid. Moreover, pasteurization of the milk affected the changes in the total nitrogen content of the cheese, and the effect was rather more noticeable when cheese was held at room rather than at low temperature. This is in accordance with the slower ripening which is normal in other varieties of cheese when made from pasteurized milk.

Chemical composition of whey draining from raw and pasteurized separated milk cheese

(*a*) *Total nitrogen content of whey.* Table 3 shows the minimum, maximum and average total nitrogen content of whey from raw and pasteurized skim-milk cheese. When cheese was held at room temperature the total nitrogen content of the whey increased all through the storage period. This is to be expected as a result of ripening and the subsequent passage of the protein degradation products into the whey.

With cheese held at the low temperature the total nitrogen of the whey again increased after the first month, probably due partly to the process of ripening, as before, and partly to the absorption by the cheese of some of the outside moisture which occurred after 1 month. The decrease in the total nitrogen of the whey which followed this increase, although very small, is apparent, and is due to the increase in the moisture draining out of the cheese which was observed after low-temperature storage for 2 months.

Table 3. *Total nitrogen content and acidity of whey from cheese made from raw and pasteurized milk and stored at room and at low temperature for* 1 *and* 2 *months*

			Storage at room temperature				Storage at low temperature			
	Initial		1 month		2 months		1 month		2 months	
Property	R	P	R	P	R	P	R	P	R	P
					Total nitrogen content					
Minimum	0·1281	0·1152	0·2212	0·2066	0·2282	0·2112	0·1506	0·1382	0·1382	0·1312
Maximum	0·1450	0·1380	0·2618	0·2324	0·2860	0·2341	0·1899	0·1860	0·1630	0·1690
Average	0·1370	0·1266	0·2475	0·2176	0·2600	0·2270	0·1659	0·1533	0·1515	0·1486
					Acidity					
Minimum	0·20	0·14	1·22	1·30	2·16	1·53	0·48	0·25	0·44	0·32
Maximum	0·36	0·21	1·98	1·66	2·39	2·13	0·86	0·54	0·87	0·70
Average	0·29	0·175	1·625	1·45	2·27	2·02	0·635	0·355	0·65	0·53

R = Cheese made from raw milk. P = Cheese made from pasteurized milk.

In general, the increase in the total nitrogen of whey was higher when cheese was held at room temperature than at low temperature; the ripening process is quicker at room temperature. Further, the extent of the increase in the total nitrogen of whey was slightly higher in the whey of the raw milk cheese than in that of the pasteurized milk cheese held at room temperature. There was no appreciable difference in the nitrogen content of the two wheys when cheese was held at low temperature.

(*b*) *Acidity of whey.* Table 3 also shows the minimum, maximum and average acidities of the various wheys. The initial acidity of whey from raw milk cheese was higher than that of whey from pasteurized milk cheese, due no doubt to reduction of acid-producing organisms by pasteurization. Consequently, the rate of increase in acidity was higher in the whey of raw milk cheese. This difference in the rates of increase in acidity occurred whether the cheese was held at room or at low temperature, although the acidity increase was more pronounced at the former. The effect of the temperature of storage was more marked than the effect of pasteurization.

SUMMARY AND CONCLUSIONS

1. Four batches of soft (Karish) cheese were made from raw and from heat-treated milk containing 1·5% fat and 7% added salt. The cheeses were sealed in tins in contact with their own whey and examined after storage for 1 month and 2 months at room temperature and at 4–6° C.

2. Cheese stored at room temperature lost weight throughout the 2 months, but cheese stored at the low temperature gained weight after 1 month and lost weight after 2 months. Parallel changes occurred in the moisture content and salt content of the cheese.

3. The fat-in-dry-matter content of the cheese increased with room temperature storage, but at 4–6° C. it decreased after 1 month and increased after 2 months.

4. Pasteurization of the milk increased the yield of cheese and the flavour, body and texture were better than that of raw milk cheese stored at the same temperature. The effect of low-temperature storage was, however, greater than that of pasteurization.

5. The total nitrogen content of the cheese decreased throughout storage, particularly at room temperature. The decrease was smaller with pasteurized milk cheese which ripened more slowly. The total nitrogen content of the whey increased throughout storage at room temperature, but at 4–6° C. it increased after one month and decreased slightly after 2 months. The increases were greatest at room temperature and in wheys from raw milk cheese.

6. The initial acidities and also the subsequent rates of increase were higher in wheys from raw milk cheese.

7. The results are discussed in detail.

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619.2

REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION E. DISEASES OF DAIRY CATTLE

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I. MASTITIS

A. *Introduction*

To introduce the section on mastitis, two papers are referred to which stress the importance of subclinical mastitis as a cause of loss to the dairying industry. In an appraisal of the present-day importance of mastitis in Britain, Wilson (1) states that progress in the control of mastitis in dairy cows has not been so great as is popularly believed, and although mastitis caused by *Streptococcus agalactiae* has been much reduced in British herds by the general use of antibiotics, the disease is still prevalent and subclinical cases are largely ignored. Other forms of mastitis which do not respond to therapy have assumed a greater role, and will have to be dealt with increasingly in the future. Wilson points out that the profit margin for milk production is narrowing, and that economically the loss due to udder damage is becoming increasingly important. In the second of the two papers Murphy (2), discussing the struggle for the understanding of mastitis in the U.S.A., points out that there is much confusion from the many terms and classifications used in connexion with mastitis. He states, for example, that there are twenty or more kinds of infection causing mastitis, whereas only four of these kinds (*Str. agalactiae*, other streptococci, staphylococci and bacilli) account for 99% of all mastitis. Each of these four occurs in three forms, subclinical, mild clinical and severe clinical. The subclinical form can be recognized only by laboratory tests, and, therefore, it is not generally realized that many diseased udders remain undetected. Almost all treatment is for clinical types only. Estimates of the loss due to mastitis have also been given. Basing their figures on a 33% incidence of mastitis which they claim to find in the main dairying district in Scotland, Laing & Malcolm (3) estimate the total annual loss due to mastitis in Great Britain to be about £19,000,000. In the U.S.A. the annual loss has been estimated by Pickard and quoted by Hodges (4) to be 226 million dollars.

* The years covered by this review are mainly 1956 and 1957.

B. *Treatment*

Important studies of the distribution of penicillin in the udder following intramammary injection are reported by Ullberg, Hansson & Funke (5,6). They made autoradiographic studies after injecting radioactive penicillin labelled with isotope ^{35}S. In mastitic udders of both cows and goats the drug was usually unevenly distributed with regions of varying size showing little or none. Essentially the same results were obtained whether the penicillin was suspended in oil or in aqueous solution. The accessibility of the tissues to the drug depended mainly upon clear passage in the milk ducts. This work confirms that the degree of penetration of penicillin into the udder is similar to that found previously for dyes and vegetable black. Papers referring to trials with various substances in attempts to obtain better penetration of antibiotics have been published. Sansoè (7) has tried the addition of hyaluronidase to antibiotics in the treatment of streptococcal mastitis. He reports the successful treatment of twenty quarters of eighteen cows, but is of the opinion that better results are obtained when additional hyaluronidase was injected 24 hr. before the antibiotics to effect preliminary dispersion of pus. The use of proteolytic enzymes is reported by Jordan (8). He used streptokinase-streptodornase, papain, crystalline trypsin and stabilized crystalline trypsin in the treatment of summer mastitis and obtained the best results with the last-named substance. It was reported in the previous review of this series (9) that in carefully controlled experiments it was shown that the intravenous injection of oxytocin, the hormone responsible for milk ejection, had no effect on the course of experimental mastitis produced by *Str. agalactiae*. Heidenreich (10), however, reports that the intravenous injection of 30–60 units of oxytocin, before therapy with antibiotics and sulphonamides, was completely successful in curing forty-four out of fifty cows suffering from chronic mastitis.

The treatment of streptococcal mastitis with antibiotics and synthetic antihistamines is reported by Ferimi, Sena & Sansoè (11) and by Visconti (12). They used 100,000 units of penicillin and 25 mg. of synthetic antihistamine. In view of the fact that certain products commonly used for the treatment of mastitis incite inflammation when infused into normal and infected udders, Foley (13) added the anti-inflammatory steroid prednisone acetate to penicillin and streptomycin which promptly checked this reaction. In a further paper (14) he reports that this substance reduced the rises in leucocyte count caused by the injection of 25 ml. of physiological saline into a quarter. He found that 4–5 mg. of prednisone or its acetate with streptomycin and penicillin caused a more rapid reduction of inflammation than the antibiotics alone. The same author (15) has also tried the effect of intramuscular injection of prednisone in the treatment of mastitis due to *Str. agalactiae*. Injection of 100 mg. into cows with quarters infected with *Str. agalactiae* caused a marked drop in the leucocyte count and in the pH of the milk. Intramuscular injection of prednisone plus penicillin caused a marked drop in the leucocyte count, and also in the number of streptococci in the milk but both increased again later. If, however, prednisone alone or with antibiotic were injected into the quarter as well as into the muscle the infection was eliminated. Martini (16) discusses the use of adrenocortical hormones in the treatment of acute mastitis and mammary oedema. It is reported by Hindson (17) that the use of ATCH with local and general antibiotic treatment considerably reduces inflammation and fibrosis.

A series of papers from Italy give details of treatment of *Str. agalactiae* infection with penicillin. Nai & Petrillo (18) report on the treatment of more than a 1000 quarters with penicillin in oil. Of quarters which showed no clinical changes before treatment 89% showed complete recovery, but in those that were clinically affected only 58% recovered. Petrillo (19) gives recovery percentages for 5000 quarters. One infusion of 100,000 units of penicillin as an oily suspension in aluminium monostearate cleared the infection in 69% of the quarters which showed no clinical signs. This figure was increased to 84% if the infusion was repeated in 3 days. The figures for penicillin in aqueous suspension were much lower, 49 and 55%, respectively. In previously untreated clinical quarters, 100,000 units of penicillin in oily suspension repeated after 8 days gave 54–60% recovery. Intraparenchymal injections of 1 mega unit of penicillin gave 85% recovery. In this work 11% of the quarters did not respond to treatment, and in a further paper Petrillo &

Vallis(20) give details of additional treatment of these quarters which included five to ten applications of ultrasonic treatment in addition to the methods given in the previous paper. An account of a programme of mastitis control involving 3358 cows in sixty-four herds is given by Nai(21). In twelve herds given no treatment infection with *Str. agalactiae* remained unchanged at 66% over an 18-month period. In two herds with an infection rate of 66% all quarters were infused with 100,000 units of penicillin at the beginning of the dry period. In 18 months the infection rate was 55%. In forty-seven herds control by isolation of infected cows and initial infusion of 100,000 units of penicillin per quarter, followed by further treatment if necessary reduced the infection rate from 64 to 12% in 6 months and it remained at this level for a year. In the remaining three herds similar success was obtained in the first 6 months, but the infection rate rose thereafter due to cessation of control measures.

In a campaign against streptococcal mastitis lasting 2 years in Samsoe in Denmark, Livoni(22) states that of 484 herds, 415 were freed from infection by treatment and the herds remained free unless infected animals were reintroduced. He stresses the need for reliable methods of diagnosis and discusses the advisability of treating only infected quarters. He advocates wider stalls for cows (1 m./cow) to reduce the risk of tramped teats. Livoni & Fórgensen(23) examined individual quarter samples of milk from 3576 cows in Jutland (Denmark) in 1947 and from 3916 cows in 1955. During this period penicillin treatment of mastitis was extensively practised. The result was that the incidence of *Str. agalactiae* infection decreased from 20·6 to 7·5%, but the incidence of other streptococcal infections increased from 3·8 to 22·6%. A 2-year campaign against mastitis in a co-operative dairy district of Sjaelland on 1300 cows is reported by Klastrup(24). Though the number of infected herds was reduced by approximately 10% mainly through the eradication of streptococci of groups B, C, G and L the level of what he terms 'constitutional' mastitis remained fairly constant in spite of treatment. McDonald(25) is of the opinion that 25,000 units of procaine penicillin once a day for 3 days is satisfactory as a general recommendation for the treatment of mastitis due to *Str. agalactiae*.

Tee(26) reports that a survey in Dorset in 1954 showed that 9% of 120 herd-milk samples contained penicillin-resistant strains of staphylococci, whereas a similar survey in 1956 of the same herds showed the percentage to be 37. He suggests that the widespread use of penicillin in the treatment of mastitis is largely responsible for this.

In the period under review very little has been reported about the use of antibiotics other than penicillin. Linssen(27) reports a higher percentage of cures with a reduction in the period of treatment if a 15 ml. dose of oxytetracycline was diluted with 100–200 ml. of water before infusion into the udder. Barnes(28) gives milk levels of oxytetracycline following local and parenteral administration. A single infusion of 400 mg. was detectable for 24 hr. or longer, and repeated infusions showed detectable levels for at least 24 hr. after the last infusion, but they did not indicate any cumulative effect of either concentration or the time for which they were present. Similar results were obtained with 800 mg. doses. Marked variations in concentrations were found in different quarters of the same cow and in different cows in the same production group, but there was very little difference in the length of time for which the antibiotic was detectable. Intramuscular or intravenous injection of 1 g. doses showed no detectable amounts in the milk. Schipper & Petersen(29), however, found therapeutic levels in the milk following 2 g. given intravenously, but not after intramuscular injection. Combined chloramphenicol-streptomycin therapy in mastitis caused by *Str. agalactiae* has been used by Petrillo(30) on fifty-nine quarters. Treatment was successful in 79% of quarters which had had no previous treatment, and in 69% of quarters which had failed to respond to previous treatment. Simon & Reese(31) found that 1·0 g. of SMP-1, a derivative of chloramphenicol, may be as effective as 500,000 units of crystalline penicillin for mastitis attributed to coagulase-positive staphylococci. Barr, Harris & Carman(32) were successful in treating eight out of nine cows showing clinical staphylococcal mastitis. They gave intramuscular injections of neomycin sulphate (1 g.) polymyxin B sulphate (1,000,000 units) in aqueous solution each day for 2 days. The animals had previously been treated unsuccessfully with other antibiotics.

Considerable interest has been shown in the treatment of coliform mastitis. Intramammary infusion of 50–100 ml. of 'Supronal' every 12 hr. for 3 days, either alone or supported by antibiotics for the treatment of coliform mastitis, is reported by Linssen (33). He obtained 80% bacteriological cures of 120 cases in the year 1954. Aehnelt & Krause (34) found that 'Supronal', oxytetracycline, chlortetracycline or leucomycin gave satisfactory results against coliform mastitis, but suggest that the sensitivity of the organisms should be tested before treatment. Bellani (35) successfully treated mild cases of acute coliform mastitis with streptomycin and mepyramine maleate. It has been reported by Fey (36) that of 136 strains of coliform organisms, which included *Klebsiella*, all were resistant *in vitro* to penicillin, but were sensitive to sulphathiazole, sulphadimidine, streptomycin and the tetracyclines. White (37) considers that due to the multiplicity of strains of *Klebsiella* and to their varying drug sensitivity, the choice of drug for treatment must depend on sensitivity tests. Easterbrooks & Plastridge (38) found sulphamethazine satisfactory for treatment of *Klebsiella* mastitis if used promptly. In cases where this treatment fails, streptomycin is indicated, but if both these fail the use of the tetracyclines or neomycin should be considered. Neomycin and polymyxin B sulphate have been found to be most effective against *Klebsiella* mastitis by Hinze (39).

In nine cows experimentally infected with *Corynebacterium pyogenes*, Bulling (40) reports that the condition responded favourably to the instillation of oxytetracycline and polymyxin B sulphate as an ointment.

Petrillo (41) reports that intramammary injection of 'Hibitane' in an oily base cured 74–86% of quarters affected with *Str. agalactiae* mastitis with no obvious clinical lesions and 45–59% of quarters clinically infected. He also reports (42) that 0·5 g. of 'Hibitane' proved harmless when infused into the udder of lactating and dry cows, but that 1 g. sometimes caused inflammatory reactions. Swartling & Petersson (43) found that milk from cows treated with 'Hibitane' was unsuitable for cheese-making for at least 2 days. Drury (44) states that Glyoxylide 6x has no value in the treatment of bovine mastitis caused by *Str. agalactiae* or *Staphylococcus aureus*. The use of nitrogen mustard in the treatment of chronic mastitis is reported by Senze & Samborski (45). Intravenous injections were given daily (10–25 mg. in normal saline) to thirteen cows, ten of which were said to show complete recovery after three to five injections. Murdane & Mathews (46) found that intramammary injection of crystalline potassium penicillin in sterile distilled water caused severe local reactions, leading to lower yields of milk containing thick clotted secretions and having high leucocyte counts. Since injection of penicillin of the same batch in other vehicles did not cause these reactions they concluded that the distilled water was not free from pyrogens and was therefore an unsuitable vehicle.

A special syringe for the treatment of mastitis is described by Neumann & Schierholz (47). The material to be injected into the teat is supplied by the manufacturers in cylindrical containers, each of which fits inside the outer casing of the syringe and acts as a plunger. Detachable teat canulas made of plastic, which are discarded after use, are supplied. In a report from the U.S.S.R. Bakhtov (48) claims that the application of cold clay poultices combined with udder massage and frequent removal of secretions gives good results in the treatment of acute mastitis. Ample farm labour would be required to use this method of treatment.

Various *in vitro* studies are reported. Alström (49) found penicillin effective against streptococci and staphylococci, but not against *Eserichia coli* and *Pseudomonas pyocyanea*. The addition of certain sulphonamides or streptomycin produced some effect against *E. coli* but not against *Ps. pyocyanea*. He also tested (50) ten antibiotics, two nitrofurazone derivatives and 'Hibitane' against the above organisms. He found penicillin the most effective against Gram-positive organisms and the tetracyclines against Gram-negative organisms. The results were the same in milk as in blood-agar cultures. Antagonism and synergism between combinations of antibiotics used in the treatment of staphylococcal infections have been studied *in vitro* by Manten (51). Combinations of streptomycin with penicillin, chloramphenicol, the tetracyclines or erythromycin were usually synergistic. Combinations of penicillin with chloramphenicol, the tetracyclines or erythromycin were usually antagonistic. A study by means of the microbiophoto-

meter of the *in vitro* activity of spiromycin associated with other antibiotics on the multiplication of *Staph. aureus* strain L-1 has been made by Mathieu & Faguet (52). They recorded bacterial growth curves and found a cumulative effect with chloramphenicol and a synergistic effect with erythromycin and with the tetracyclines. Penicillin and streptomycin were more active alone than when associated with spiromycin, but the action of spiromycin was not modified by the presence of the other two. Sensitivity tests to penicillin, streptomycin, oxytetracycline and chloramphenicol of organisms from cases of mastitis seen in general practice have been made by Hemsley (53). Resistance to penicillin was shown by two of four organisms isolated from cases previously treated with penicillin. Coliform organisms were sensitive to oxytetracycline and chloramphenicol. In the infection in one quarter, possibly due to *Str. uberis*, the organism was resistant to all the above-mentioned antibiotics, but the infection cleared with intramammary injection of sulphathiazole. The *in vitro* diffusion of antibiotics through fibrin membranes is reported by Watson (54). Penicillin diffused rapidly reaching equilibrium in 5 hr. The passage of chloramphenicol and chlortetracycline was much less rapid, resulting in one-fifth to two-fifths, respectively, of theoretical equilibrium after 48 hr. Erythromycin and spiromycin diffused across the membrane with still greater difficulty. A new rapid plate method for determining antibiotic sensitivity is described by Pital, Disque & Leise (55). Impregnated disks are placed on seeded plates and incubated at 37° C. for a short time. After removal of the disks 0·6 ml. of 0·05% solution of resazurin is poured over the plates and allowed to diffuse for 10 min. The plates are returned to the incubator and in a relatively short time the inhibition zones are clearly indicated by colour contrast. The tests are complete in just over 2 hr. for staphylococci. Chloramphenicol, chlortetracycline, oxytetracycline and penicillin were tested by this method. Linzenmeier (56) gives a method of applying inhibitor substances to agar plates. Filter-paper disks are cut to the shape of cog-wheels each tooth of which is impregnated with an inhibitory substance to be tested.

C. *Streptococcal mastitis*

Bacteriology. A number of papers refer to the CAMP test. A comparison of the results obtained by examining quarter milk samples and bulk milk by means of the CAMP test has been made by Klastrup & Pedersen (57). Infection with streptococci of groups B, C, G and L was established in twenty-eight herds by udder samples and in twenty-two herds by bulk samples, which is equivalent to 78·5% agreement. In this series the bulk milk was examined on only two occasions. In a further series the bulk milk and quarter samples were examined every day for 11 days and 92% agreement was obtained. Obiger (58) considers that the best method for routine diagnosis of group B streptococci in milk samples is to streak the sample on to T.K.T. medium (thallium sulphate, crystal violet, β-staphylococcal toxin blood-agar), pick off the typical streptococcal colonies and identify the group B streptococci by means of the CAMP test. In a study of 422 strains of streptococci obtained from cases of mastitis Seelemann & Obiger (59) confirmed that the CAMP reaction is, in general, specific for group B streptococci, although a few strains of group E and P produced a similar cup or wedge-shaped area of haemolysis. The use of T.K.T. medium was of special value in detecting mastitis streptococci in bulk milk.

For routine examination of milk samples for *Str. agalactiae*, de Vries (60) enriches the samples for 20–40 hr. at 37° C. Those giving a positive reaction in the Hotis test are inoculated onto Edward's medium and typical colonies picked off this medium onto aesculin blood agar for examination by the CAMP test. Nai & Negretti (61) found that of 1000 strains of *Str. agalactiae* that were positive to the CAMP test, 580 gave a positive reaction to a rapid slide agglutination test. Owing to self-agglutination, tests on the remainder were not possible. In a second series of 1000 strains the number of self-agglutinating strains was reduced to 55 by the addition of 2% glycerin to Wilson & Slavin's medium and by reducing the incubation temperature to 30° C. The remaining 945 samples were positive to the agglutination test. Pulsford (62) found that a new spot-test based on the CAMP reaction was simpler and cheaper than the Hotis test for the detection of *Str. agalactiae* in milk samples. Throop, Swanson & Mundt (63) compared the results of the Hotis test with the identification of streptococci by other means. They found the test to be

82% efficient for the identification of *Str. agalactiae*, but it was not good for the identification of *Str. dysgalactiae* or *Str. uberis*. Negretti & Langé (64) found that of 1143 strains of *Str. agalactiae* isolated from cows in various regions of Italy the following serological types in order of frequency were identified: 1*b*, 7*a*, 6*a*, 1*e*, 1*c*, 3*a*, 2*a*, 4*b*, 1*a*, 1*f*, 3*e*, 3*b*, 3*c*. There were also ten strains similar to the Pat 85 strain not yet classified. They observed that several types may be concerned in infection in the same quarter of the udder.

Experimental. In the twelfth paper in the series on experimental streptococcal mastitis from Compton, Bracewell & Pattison (65) report on the testing of *Str. agalactiae* vaccine under field conditions. Vaccinated and unvaccinated cattle were exposed for 58 weeks to the risk of infection with the same strain as was used to make the vaccine. Methods of spreading the organism were: (*a*) for the first 20 weeks until the end of the first lactation by donor cows, and (*b*) for 32 weeks of the second lactation by dipping the cows teats three times a week in a culture of the organism. Infection occurred in two out of nineteen vaccinated cows (two quarters) and in seven out of fifteen non-vaccinated cows (thirteen quarters). This result shows a minimal level of statistical significance in favour of the vaccinated animals. They conclude on the basis of this and previous experiments that the measurable protection given by the method of *Str. agalactiae* vaccination was too small to be likely to play an important part in controlling *Str. agalactiae* mastitis in the field. They point out, however, that as streptococci, which as a group are well recognized as being poor antigens, have been shown to have some small beneficial activity, further immunological studies with other organisms that are associated with mastitis would seem to be well worthwhile. Spencer & Simon (66) found that young lactating cows developed cutaneous hypersensitivity to *Str. agalactiae* after repeated injections of the living organisms. Subsequently, the animals were apparently less susceptible to intramammary infusion of *Str. agalactiae* than the control animals. Experiments are described by Karlshøj & Pedersen (67) in which cows were injected with various doses of milk culture of a strain of group B streptococcus which had been freeze-dried shortly after isolation. The milk used for growing the culture was taken from the cow on which the test was to be made and was twice heated to 70° C. for 30 min. with a 24 hr. interval. Even small doses were able to induce persistent infections if the organism had been adapted to growth in the milk of the cow. An interesting report on the continuation of infection with *Str. agalactiae* through the dry period in the cow is given by Howell (68). Seven cows which were dried off when known to be infected in one quarter with one of two strains of the organism, yielded the identical strains from the same quarters on coming into lactation after dry periods lasting from 12 to 53 weeks.

Yield. Hale, Plastridge & Williams (69) have compared the milk yields in 328 herds in the Connecticut Dairy Herd Improvement Association, during an average interval of 5 years. Of 120 herds in which *Str. agalactiae* was not present, twenty-four became infected and the average yield per cow dropped 10·2%, whereas of 208 infected herds ninety-one became free from infection and the average yield per cow increased by 14·8%. Noorlander & Schalm (70) give graphs to show that graphical recording of a cow's milk production, based on the weight of milk produced in a 24 hr. period once a month, can provide a basis for evaluating the effect of mastitis on yield and for the measure of improvement following control and treatment.

D. *Staphylococcal mastitis*

Typing. The characters and distribution of certain strains of staphylococci are described by Edwards & Rippon (71). From the milk of cows in three herds at Compton and from nineteen herds in the surrounding district 395 strains were obtained. The majority were from routine herd tests, and a smaller number from cows with clinical mastitis. By phage-typing methods 381 of the strains could be divided into five main types.

Type 1. Lysed predominantly by phage 42D.

Type 2. Lysed predominantly by strong filtrates of 3A, 3B, 3C and 71.

Type 3. Lysed predominantly by phage 52B.

Types 4 and 5. Lysed predominantly by phages 52A or 78.

Compared with coagulase production against rabbit and sheep plasma these types gave a constant pattern and this test formed a useful method of confirming the identity of the phage types. Of 120 strains tested, fourteen strains were resistant to penicillin. It is interesting to note that all belonged to type 3. Barnum & Fuller(72), using forty-two lysogenic cultures, phage-typed 81% of 591 strains of haemolytic staphylococci isolated from the udder. They differentiated five groups each including strains from normal udders and from cases of mastitis. In general the organisms of one phage group caused all the mastitis in a given herd. A method of phage typing more or less combining three previously reported methods is described by Seto & Wilson(73). Of 379 coagulase-positive β-haemolytic micrococci from cases of bovine mastitis, 93% were sensitive to one or more of seven selected phages. None of fifty coagulase-negative cultures was sensitive to any of the phages. Thörne(74) isolated 1480 strains of staphylococci from quarters with clinical mastitis and subjected them to the coagulase test (K), mannitol fermentation (M) and the CAMP reaction (C). The strains were differentiated as follows:

Type	Reactions	No. of strains	Haemolysis
I	Positive to K, M and C	271	Distinct
II	Positive to K and M	124	Distinct
III	Positive to K	246	Indistinct
IV	Negative to K, M and C	839	Indistinct

He considers that mastitis in herds in which type IV is dominant is due mainly to environmental factors, but that where type I predominates the staphylococcus itself is an important aetiological factor. He also tested forty-eight strains by intracutaneous injection in cattle(75). Strains of type I showed the severest reactions and those of type IV the mildest. The same author(76) has studied the haemolytic properties of coagulase-negative staphylococci isolated from milk. The cultural characters of thirty-nine strains of staphylococci isolated from cases of mastitis are described by Worseck(77). The reactions studied were pigment formation, haemolysis, liquefaction of gelatin, fermentation of mannite, coagulation of rabbit plasma and production of dermotoxin when inoculated into rabbits. Six strains were resistant to penicillin. Attempts to identify bovine coagulase-positive staphylococci by precipitation techniques have been made by Pattison & Matthews(78), but because of extensive sharing of precipitating antigens among strains of widely different origin and because many sera defied all attempts at absorption the work was unsuccessful. They point out that in work on mastitis concerned with the behaviour of staphylococci as antigens, it is required not only to identify them but also to relate them to serological responses. A flocculation test is described that was of some use in separating strains, but it failed to detect soluble as well as insoluble antigen-antibody combinations. Pattison & Matthews give preliminary observations on the application to bovine staphylococci of the diffusion plate technique of Ouchterlony(79). This technique indicated that constant serological differences existed between groups of strains, but more work is necessary before any sort of classification of bovine coagulase-positive staphylococci could be based on this test though the formulation of such a classification may not be impossible. Chabbert & Debruge(80) report that spontaneously resistant strains of staphylococci isolated from human patients may undergo 'reverse mutation' which can yield a very high proportion of susceptible variants. The resistant strains produce in the usual media a substance, diffusable in agar, able to inhibit the action of doses of chloramphenicol that would prevent the multiplication of susceptible strains.

Before taking milk samples Schalm & Lasmanis(81) used 1 oz./gal. of sodium hypochlorite to 'sterilize' udders. Samples were incubated for 18–20 hr. at 37° C. at the farm and then refrigerated and sent 60 miles to the laboratory. Micrococci comprised 70% of the organisms isolated from the fore milk, regardless of the lactation age of the cow. The occurrence of coagulase-positive organisms increased with age, however, with a proportional decrease in coagulase-negative micrococci. As has been reported in a previous review of this series(82) the use of enrichment methods is not satisfactory for estimating the incidence of staphylococcal infection.

Experimental. A preliminary report on the immunization of animals against *Micrococcus*

pyogenes is given by Spencer, Stewart & Lasmanis (83). They tried formalized culture in mineral oil and also in aqueous solution suspended in aluminium hydroxide. High antihaemolysin titres, which persisted for 14 weeks, were obtained with both methods. They challenged sixteen quarters with viable cultures and the reaction was less than in five quarters of unvaccinated cows. A chronic infection developed in nine of the sixteen quarters. Using two strains of haemolytic coagulase-positive staphylococci Klastrup (84) made eighteen inoculations into eight quarters of three cows in their first lactation. By first allowing the staphylococci to enter the growth phase in milk withdrawn from the quarter to be inoculated, he produced seven cases of mastitis with a small number of organisms (1–139 cocci per quarter). The degree of reaction varied from subclinical mastitis to acute mastitis with loss of the quarter.

E. *Mastitis caused by organisms other than streptococci and staphylococci*

A brief account of the pathology of necrotic mastitis in cows caused by *E. coli* is given by Renk (85). Redaelli (86) reports etiological, pathological and clinical observations of coliform mastitis in fifty cows. He describes two syndromes, a chronic form which affects only the udder and lasts for not more than a few days and an acute form in which there is fever, diarrhoea and paralysis as well as changes in the milk with possible drying off of the quarter. The strains of organisms isolated from the two forms differed in serological and biochemical characters. Fey (36) found that, of 136 strains isolated from coliform mastitis in cows, 113 were *E. coli*, twenty were *Klebsiella*, one was *Proteus morgani* and two were unclassified. Necrosis in laboratory animals was caused by 30% of the strains and it is suggested that this type is responsible for necrotizing mastitis. An outbreak of mastitis in cows and death of calves due to an infection with *Klebsiella* is reported by White (37). There were four cases of mastitis and the infection was spread to calves by way of the infected milk. Crews (87) gives the cultural properties of *Klebsiella* organisms derived from cases of bovine mastitis which may be summed up as follows: they were lactose-fermenting, Gram-negative, non-sporulating, non-motile and capsulated; they did not liquefy gelatin and were indole negative, methyl red negative and V.P. negative. They utilized citrate, reduced nitrates, split urea, but did not produce H_2S. He considers the organism from the udder to be a variant of *Klebsiella pneumoniae*. A report on five field cases and four experimental cases of acute *Klebsiella* mastitis is given by Easterbrooks & Plastridge (38). Mastitis caused by capsulated coliform organisms and by *Klebsiella* were shown to be the same. The authors point out that the public health significance of *Klebsiella* mastitis is yet to be determined. Hinze (39) considers that any case of mastitis caused by a Gram-negative capsulated organism which is not readily identified by the usual cultural and biochemical methods should be regarded as being caused by *Klebsiella*.

An account of acute mastitis due to infection with *Pseudomonas pyocyanea* in two cows which had recovered from grass tetany is given by Lambardt (88). He suggests that the resistance of the animals may have been weakened. Mahoney & Armstrong (89) demonstrated that the water supply on a farm was contaminated by *Ps. pyocyanea*. It is thought that mastitis in a cow caused by this organism was the result of washing milking equipment with the water. Neumann & Seelig (90) isolated *Ps. pyocyanea* from the udder secretions and udder tissue of a cow with acute haemorrhagic mastitis. Mastitis and generalized lesions in the thorax and abdomen of a cow caused by *Ps. pyocyanea* is reported by Winter & O'Connor (91).

There are reports of other organisms causing mastitis. Mastitis associated with *Bacillus cereus* is described by Brown & Scherer (92); with *Pasteurella haemolytica* by Maplesden & Carter (93); with *Cryptococcus neoformans* by Ségretain, Verge, Drieux, Mariat, Paraf, Labie & Théron (94) and with *Clostridium welchii* by Bühlmann & Boller (95). An organism of the Nocardia group was isolated by Pier, Gray & Fossatti (96) from a dairy herd in the coastal area of California. Of twenty-nine animals, whose milk was examined on one to three occasions, sixteen shed this organism. Clinical signs varied from local inflammation to a severe systemic reaction. Examination of one udder *post mortem* showed thick-walled lesions, 2·5–5·0 cm. in diameter. Cultural and

histological examination of these lesions is not mentioned. Jungerman (97) reports the repeated recovery of *Nocardia asteroides* from one quarter of a cow. The milk was watery and contained yellowish and whitish flakes and clots, and the quarter became progressively filled with scar tissue. From a quarter with chronic mastitis of 3 months standing, de Vries & Strikwerda (98, 99) isolated *Erysipalothrix monocytogenes*. In twenty-seven cows on a farm in the Crimea mastitis, mainly in one quarter of the udder, was stated by Semenchuk & Skruipnik (100) to have been caused by *Candida tropicalis*, *C. stellaroides* and *C. peracrusei*. Infection was accompanied by fever, depression and in some cases loss of appetite. The condition lasted for periods ranging from 3 days to 4 weeks and was associated with the feeding of melon and beet. An outbreak of mycotic mastitis associated with the dry storage of teat-cup liners is reported by Simon & Hall (101). Fungi resembling *Candida* were isolated from the liners. The liners were then stored in 0·5% lye and no fresh cases occurred. *Candida* species were isolated by Kauker (102) from four cases of mastitis, *Saccharomyces fragilis* from two cases and *Hansenulo anomala* from one case. According to this author, mastitis caused by yeasts is characterized by enlargement and oedema of the udder, enlargement of the supra-mammary lymph node, fever and greyish white, slimy milk.

F. *Diagnosis*

Numerous papers on the Whiteside test have been published during the period under review. Obiger (103) has compared the results obtained by that test with results given by the leucocyte count, the catalase test, the determination of the chloride content of the milk and the bacterial count of the milk. In 2000 quarter milk samples he got 83, 86, 79 and 83% agreement, respectively. The percentages for cow-milk samples were similar, but for 1000 churn-milk samples they were 74, 70, 75 and 67, respectively. Though this test detected only 202 out of 489 samples containing streptococci, it was, nevertheless, considered by Obiger to be a useful aid to the detection of mastitis. Jensen, Schipper & Aschbacher (104) are of the opinion that bulk milk with a strong positive Whiteside reaction can be suspected of coming from a herd where mastitis is a problem. Andersen (105) found that numerous comparative tests on mixed milk made during a 4–5-year period showed that the Whiteside test furnished better general information about infection than did the catalase or the blood-toxin agar tests, and he recommends its adoption as the principal test for mastitis. Procedures for the use of the Whiteside test on milk in the laboratory or byre are described by Schalm, Gray & Noorlander (106). About 10 ml. of fore-milk from each quarter of the udder are added to four tubes with 2 ml. of 4% sodium hydroxide and a drop of brom-cresol purple in each tube. The tubes are rapidly inverted several times and the glass above the milk inspected from particulate material the presence of which indicates a positive reaction. A large-scale scheme for testing cows in the Sacramento county, California, for Whiteside test activity is described by Schalm, Pier, Gray & Noorlander (107). The 'field' Whiteside test as described above and the plate Whiteside test were compared and the latter was found to be more efficient. Milk from a herd of 106 cows showed agreement between both methods in 91% of the samples. Experiments and observations leading to the development of the California Mastitis Test (CMT) are given by Schalm & Noorlander (108). The test was developed from the Whiteside test. The Whiteside test was found to depend on the amount of cream in the milk sample for the degree of reaction, whereas the CMT does not. The clotting is of the added agent itself. They tried a number of surface-active agents, and found that the reaction was seen when milk of high cell count was brought into contact with any of the following: long-chain fatty acids, alkyl sulphates, alkyl sulphonates, alkyl amylsulphates or alkyl amylsulphonates. The authors do not name the actual agent used, but in Britain a detergent selling under the commercial name of 'Teepol' has been found to be satisfactory. A white plastic paddle has been designed with four receptacles, into which the milk is drawn directly from the respective teat. The test solution is added in a quantity estimated to be equal to that of the milk in each cup. The reactions occur and are graded while the milk and reagent are mixed by a gentle circular motion of the paddle. The cell count of the milk is indicated by the degree of precipitation or gel formation. In a series

of 481 quarter samples of fore-milk the mean total cell count per ml. compared with the CMT score was as follows:

	CMT score				
	Negative	Trace	1	2	3
No. of samples	271	52	95	43	20
Total cells/ml. in millions	0·08	0·43	1·15	3·25	11·43
Percentage polymorphs	23	31	48	61	77

Frank & Pounden (109) compared the CMT and the Whiteside test on 2000 quarter milk samples using six ratings for each test and got a 63% agreement, but if adjoining ratings were combined the agreement was 80%. The disagreements were in the more normal samples. Investigations into the Whiteside test and the CMT have been made by Jensen (110). He found that the CMT showed a much better correlation with clinical findings than the Whiteside test.

The catalase activity of colostrum and normal milk and its significance in the detection of bovine mastitis has been studied by Nagasawa, Tanahashi & Kuzuya (111). The catalase activity of colostrum was considerably higher than that of normal milk even 1 week after calving. In cows with no history of mastitis milk giving a positive Hotis test displayed approximately twice the catalase activity that was shown by milk giving a negative Hotis test. Milk having catalase activity similar to or higher than that of a positive Hotis test milk may possibly indicate the presence of subclinical mastitis. Catalase activity in milk is caused partly by the bacteria present and partly by leucocytes. Filipovitch & Filipovitch (112) analysed the chloride content of over 9000 milk samples. Taking 0·14% as the maximum acceptable chloride content for normal milk over 1600 had a high chloride content, but only 20% of these were associated with pathologically abnormal milk. They concluded that a high chloride content does not necessarily mean mastitis, and milk from cows with acute mastitis frequently had a normal content of 0·07–0·14%.

A modification of the Breed count is described by Petrillo (113). The number of fields needed in order to count fifty cells is divided into fifty to give the number of cells per field and the figure obtained is multiplied by 300,000. This is done to avoid counting too many cells in smears of milk with high cell counts. It is probable that most laboratories have their own simpler methods of avoiding the waste of time involved in counting more cells in a smear than it is really necessary to count.

Murphy (2) refers to the muddle caused by laboratories using different methods for the diagnosis of mastitis by culture methods. Some laboratories incubate the sample first, some incubate the sample and then use selective media, whilst others refrigerate the sample until it can be plated direct on to solid media. In the reviewer's opinion the direct plating method is the only satisfactory one for the routine diagnosis of mastitis. After describing methods for diagnosing the various types of mastitis and abnormalities in milk, Seelemann & Obiger (114) advocate the adoption of standardized routine methods of diagnosis. In the examination of 165 cows with mastitis Zayanchkovskiĭ, Selunskaya & Gritskevich (115) made the following diagnoses: serous 8·5%, catarrhal 73·3%, gangrenous 9·7%, fibrous 5·5%, haemorrhagic 1·2% and others, including one case of tuberculous mastitis, 1·8%. An 'incubation liquid' described by Livoni (22) for selectively inhibiting certain bacteria is criticized by Olsen (116). He points out that crystal violet in milk becomes protein bound and is therefore not inhibitory to staphylococci.

G. *General*

Summer mastitis. A number of papers referring to the subject of summer mastitis has been published during the period under review. The Production Division of the Milk Marketing Board (117) collected information on the incidence of this condition in the years 1954 and 1955 and found that the incidence was six times higher in July and August 1954 than in the corresponding months of 1955. A survey by students 'seeing practice' similar to the survey reported in the previous review of this series (9) was made in the summer of 1955 and is reported by Stone (118). *Corynebacterium pyogenes* was isolated in pure culture from 44 of 120 samples of milk from cases of summer

mastitis, and with other organisms from a further twelve samples. These figures are similar to those reported for the year 1954. After making a detailed examination of twelve udders affected with *C. pyogenes* mastitis, Jacob (119) formed the opinion that the infection entered through the skin. Bahr (120) describes three trials in which protection against summer mastitis was attempted by the use of a fly-killing ointment on the teats of dry cows, and Aehnelt (121) describes trials made with more than 1000 dry cows by spraying with three commercial insecticides including 'Toxophane' and 'Lindane'. Hornstein, McGregor & Sullivan (122) found that after using 'Lindane' cattle sprays to which film-forming material was added to reduce the volatility, 'Lindane' concentration in the milk did not fall to 0·1 p.p.m. until 6 weeks after spraying.

Incidence of mastitis. In a survey by Withers (123) of wastage and disease in dairy herds, reference is made to the incidence of mastitis. In approximately two-thirds of the herds in the survey cases of clinical mastitis were recorded, and there was a tendency for the disease to be more prevalent in larger herds. Bratlie & Fodstad (124) obtained data from sixty veterinary surgeons covering 965 cases of acute mastitis; 9% were associated with group C streptococci; 9% with *Str. uberis*; 3% with atypical streptococci; 19% with coliform organisms; 12% with staphylococci; 12% with *C. pyogenes*; and from 13% there was no growth. Bacteriological examination of the secretions from fifty cases of mastitis made by Heidrich & Kelch (125) showed that in 32% no specific organisms were found. Of the remainder *Str. agalactiae* was found in 28%, *E. coli* in 24%, *C. pyogenes* in 8%, *Bacterium aerogenes* in 4%, *Staph. albus* in 2% and *Bacillus proteus* in 2%. Laing & Malcom (3), using the total cell count and bacteriological examination to examine 67,664 quarter samples of milk, found that non-specific mastitis constituted about 54% of affected quarters, staphylococcal mastitis about 25% and purely streptococcal infections only 15%.

In a study involving the examination of 3264 quarter samples from 816 cows on small farms in the Zagreb area, Milunović & Lajoš (126) isolated *Str. agalactiae* from fifty-one quarters (forty-one cows); *Str. dysgalactiae* from five quarters and *Str. zooepidemicus* from one quarter. A survey to determine the incidence of mastitis due to *Str. agalactiae* throughout Italy has been made by Nai & Vallis (127). They examined samples from 10,405 cows on 783 farms, and found that 43% of the farms, 29% of the cows and 6% of the quarters were infected. They found great regional variations. Little or no mastitis was observed on the small farms on acid soil where a few triple-purpose animals are kept, whereas 50% of the cows were infected in parts of Lombardy where high yielding animals are permanently housed. Hadžidedić (128) reports that in five surveys done in recent years in several provinces of Yugoslavia, mastitis due to *Str. agalactiae* was diagnosed in 5–31% of the cows examined.

Milking technique. It has been found by Moore (129) that if a comparatively large volume of plain water is used to rinse the udder the bacterial counts of freshly drawn milk are lower than they are when the udder is wiped with a cloth soaked in hypochlorite solution. The best of five methods of udder washing tried by this author, was first to rinse it with plain water (which was not re-used) and to follow this by wiping the udder with a cloth soaked with a 200 p.p.m. hypochlorite solution. Using this method hypochlorite was better than a quaternary ammonium compound. Stewart, Spencer, Lasmanis & Holliday (130) have tested the efficiency of chlorine, a quaternary ammonium compound and 'Hibitane' in teat-cup decontamination. Against organisms capable of causing mastitis chlorine appeared to be inferior to the other two compounds. 'Hibitane' had a more active bactericidal and bacteriostatic effect *in vitro* than the quaternary ammonium compound. Experiments using an artificial udder have been made by Newbould & Barnum (131). The 'udder' was contaminated with micrococci by passing infected milk through it, and the infected teat cups were dipped in the following antiseptic solutions: Diversol, Antibac (both chlorine compounds), Iosan (an iodophor) and 'Hibitane'. Dipping for 30 sec. in any of the four solutions resulted in a 90% reduction in the number of organisms on the liners. When farm trials were done with this treatment the results were not so promising. In a further paper (132) these authors report that dipping the teat cups in 'Hibitane' seemed to keep the organisms on the udder at a much lower count than did other disinfectants. This would

appear to be due to the residue of the 'Hibitane' being transferred to the teats, and since its activity is not reduced by contact with organic material, it continued to act on the organisms on the teats.

Turning to the subject of milking-machine design, Dodd, Oliver & Neave (133) have studied the effect on udder health and on milk yield when half-udders were milked with either a moulded or an extruded teat-cup liner. There was higher incidence of mastitis with the moulded liner, but the incidence of new infection was similar with both types. They found no measurable difference of erosion of the teat orifice, and neither the leucocyte counts nor the decline in milk showed a significant difference between the two groups. Porter, Miller & Skaggs (134) milked forty-four cows for alternate 90-day periods at high and low vacuum levels. They found no difference in the incidence of mastitis, fibrosis, leucocyte counts, yield or persistency. The change of levels caused a temporary uneasiness, depression of yield and increase of leucocyte count, but this only lasted for 5 days. Pier, Schalm & Hage (135) have shown by radiographic means the action of the milking machine. They draw attention to the fact that negative pressure (i.e. 'vacuum') is capable of causing injury to the tissue of the udder if it is improperly applied. Schalm & Noorlander (136) describe an experimental milking machine with modified pulsation, in that no collapsing liner is used, and with a clear plastic cylinder for the teat cup which provides visual milking.

Pathology. Macadam (137) has studied the pathology and bacteriology of bovine mastitis in relation to the cell count. He examined ninety-four quarters from twenty-four cows in their fifth or later lactations. The total cell counts were generally directly related to the extent of the pathological changes in the udder. The proportion of polymorphs in the milk generally exceeded 70% in recently inflamed quarters and was below 40% in involution in the absence of acute mastitis. Quarters infected with *C. pyogenes* and *Str. agalactiae* showed in general about twice the amount of pathological change that was observed in those infected with *Str. uberis* or coagulase-positive staphylococci and four times the amount of those infected with coagulase-negative staphylococci. The mammary pathology of 'blind' quarters has been studied by Renk (138). The diseased udders of eighty cows were examined and non-lactating quarters were found in twenty-two of them. In twenty of the twenty-eight dry quarters, cessation of lactation was caused by blockage of the sinus, resulting from proliferative inflammation, accompanied in some cases by epithelial hyperplasia. The lesions were associated with *C. pyogenes* in seven quarters; they were non-specific in five quarters and were the result of teat injury in four quarters. Detailed illustrated case reports of the histopathological examination are given.

Milk resistance. Further papers have been published on the resistance of milk samples to the activity of *Str. agalactiae in vitro*. Pounden, Frank & Krauss (139) found that milk taken immediately after turning cows out to grass was less resistant to the growth of *Str. agalactiae* than those taken before, as shown by the increase in acid production. They found that in a wet season the level of resistance fell during the summer but in a dry season it was well maintained. Pounden, Frank, Brown & Scherer (140) found that relatively high levels of resistance were maintained by milk samples from cows fed on rations containing silage, either forage-crop or 'corn'. They go so far as to claim that the change to this type of feeding is generally the reason for a reduction in the incidence of *Str. agalactiae* infection.

Composition. Three papers refer to the chemical composition of milk in relation to mastitis. Waite & Blackburn (141) examined six samples from each of 360 cows during a lactation and also 823 samples of farm milk taken at a receiving creamery. Each sample was analysed chemically and for its total and differential cell counts. The total cell counts varied from less than 10,000 to 5,000,000 per ml. and there was considerable variation in the chemical composition of samples containing similar numbers and types of cells, but as the total cell count increased to an average value of 500,000 per ml. there was a fall in the daily yield of milk per cow and a marked fall (0·25%) in solids-not-fat content. The latter was the result of a steady drop in the lactose content, the casein content being affected only when counts approached or exceeded 1,000,000 per ml. The possible reasons for the relatively small effect on chemical composition of high cell counts in some milks is discussed. McKenzie, Booker & Moore (142) report that over a 2-year period tests

of milk from an Ayrshire herd showed that more samples with high cell counts had low solids-not-fat than did samples with low cell counts. While wide variation of solids-not-fat was found at any particular level of cell count, there was a broad inverse correlation between cell count and solids-not-fat, the average solids-not-fat for cows in the same lactation being lower for those animals with high cell counts than for those with low cell counts. Defranceschi & Nani (143) found an increase in the total nitrogen of milk samples which were infected with *Str. agalactiae* and had cell counts of over 500,000 per ml. compared with milk which was not infected.

Risk to human population. A few papers on mastitis and the risk it may be to the human population have been published. Smith (144) discusses the inhibitors to the growth of staphylococci which are present in raw milk but not in heated milk. The fact that milk is kept usually at a lower temperature than is suitable for the multiplication of staphylococci and that inhibitors are present may be the reasons for the rarity of staphylococcal food poisoning from drinking milk. Jones, King, Fennell & Stone (145) report that in milk from a cow naturally infected and in milk drawn aseptically from a normal cow *Staph. aureus* grew well at 30 and 37° C., but that below 25° C. growth was so slow as to render negligible the chances of milk becoming toxic within 24 hr. In addition the presence of lactic and faecal streptococci in numbers equal or superior to those of the staphylococci appeared to restrain the growth of the staphylococci, particularly at the lower temperatures. It is concluded, therefore, that staphylococcal food poisoning is unlikely to occur unless: (1) the milk is withdrawn from the udder under very good hygienic conditions from a naturally infected udder, (2) is kept at a fairly high temperature for a sufficient length of time, (3) is not mixed with the milk of other cows to a sufficient degree to dilute the toxin below active level, and (4) is drunk raw.

H. *Conclusion to mastitis section*

Although much work has been done on mastitis during the past few years, relatively little progress appears to have been made towards reducing the magnitude of the problem. The work on treatment has been mainly a confirmation and extension of work previously reported. Under the heading of diagnosis the emphasis is still on methods for the rapid recognition of *Str. agalactiae*, though some hope of the differentiation of strains of staphylococci appears to be emerging. The importance of subclinical mastitis as a cause of economic loss is now being recognized and reports on the Whiteside test and the California Mastitis test as a means of diagnosing the condition are given. It should be remembered, however, that these tests are merely methods of making a rapid estimate of the number of cells in milk, and that a high cell count does not always indicate mastitis. It is encouraging to note the continued study of milking technique both as regards hygiene and in the design and use of the milking machine, since this is an important approach by which advances towards the prevention of mastitis will certainly be made.

II. BRUCELLOSIS

A. *Diagnosis*

Numerous papers on the subject of milk agglutination tests have been published in the period under review. The Public Health Laboratory Service reports (146) that sole reliance cannot be placed on any of the milk agglutination tests for the detection of *Brucella abortus* in milk samples. In 60–70% of samples reacting positively the organism cannot be demonstrated by guinea-pig inoculation. The tests are of most value as herd tests and in the laboratory as screening tests. Ogonowski (147) has found that milk samples from infected cows were constantly negative to the milk-ring test when the fat globules were relatively uniform and predominantly of small size, and invariably positive when the fat globules showed a marked difference in size. These findings, based on the theory of cream formation, may partly explain the occurrence of negative milk-ring test reactions in infected milk. It is concluded by McDiarmid, Findlay, Jameson, Phease, Walker, Jones & Ogonowski (148) that the milk-ring test and the plate-agglutination test are of no great value in determining the state of immunity of individual cattle that have been vaccinated

in various ways with S. 19 vaccine before breeding age and that have been exposed subsequently to a known *Br. abortus* infection. Milk from non-infected cows may give a positive response and many samples from known infected cows fail to react to these tests. They found the ring test to be more sensitive than the plate test under the conditions of their investigation. Krejčí(149) examined the effects on the milk-ring test of various factors such as the butterfat percentage, fat globule size, pH and age of the milk, temperature and dilution. After comparing the results obtained from this test with those from the serum agglutination and complement-fixation tests he considered the milk-ring test to be the most sensitive and recommends it as the most suitable for use in the control of bovine brucellosis. Mrygón(150) is of the opinion that the agglutinins in the milk are maintained at a more constant level than those in the blood. No differences were found by Genest(151) in results obtained by the milk-ring test when samples were incubated at 37° C. for 15 min. and when left at room temperature for the same period. Genest, Lahaye & Filion(152) found that the ring test, using a tetrazolium blue-stained antigen with milk or cream samples, proved efficient in detecting positive cases. Centrifuging proved to be helpful in making the test. Thus tests of samples from 1710 herds showed that a 15 min. period of incubation with antigen followed by 1 min. centrifuging at 1000 r.p.m., disclosed a higher proportion of positive samples than did a period of 60 min. incubation alone. The ring tests agreed with the serum agglutination tests in 97·9% of the herds tested. Sykes(153) reports that it is possible to produce adequately stained suspensions of *Br. abortus* for use in the milk-ring test with either neotetrazolium chloride or blue tetrazolium. The method in which these substances are used tends to be quicker and simpler than methods in which haematoxylin is used. The tetrazolium method gives easily reproducible results, and colour variation from batch to batch is minimal. The chief factors governing the production of an adequately stained antigen by means of the tetrazolium salts mentioned are the concentration of the tetrazolium (10 mg./100 ml.), the period of incubation (5 hr.), the concentration of the cells (0·2%), and the pH of the suspending medium (pH 9·5). False positive reactions to the milk-ring test were detected by de Vries(154) by repeating positive tests with milk diluted 1:4 and 1:8 with negative milk. The results were compared with those of the whey-agglutination test. This procedure was found useful when the result of the serum-agglutination test was doubtful. Samples of churn milk which were positive or doubtful to the milk-ring test, or were negative but came from herds whose milk was positive in the previous year, were subjected by Köser(155) to the slow-agglutination test. Of 9486 negative samples 7·8% were positive to the slow-agglutination test, and of 16,928 positive samples 11·9% were negative to the slow-agglutination test. From these results he concluded that the testing of churn milk by the milk-ring test was inadequate for the eradication of *Br. abortus* infection. It would be wrong, however, to presume that the slow-agglutination test is therefore adequate for this purpose. Shalash(156) reports that the results of tests on 200 buffalo milk samples showed that a haematoxylin-stained antigen can give a positive reaction when anti-abortus serum is mixed with normal milk up to a dilution rate of 1 in 40, whereas a tetrazolium-stained antigen can give a positive reaction with a dilution up to 1 in 70.

Reports of work with the whey test for brucellosis come from the U.S.A. where Cameron & Kendrick(157) found that of 161 cows excreting *Br. abortus* in the milk the blood-serum agglutination test was positive with only 135, whereas the plate-agglutination test using whey was positive with 159. In addition, a herd of cows which was vaccinated became negative to the whey test after 9 weeks, while it remained positive to the blood-serum agglutination test. It is, therefore, considered that the whey test will distinguish between vaccinated and infected animals. Cameron & Kendrick(158) stress their finding that vaccination with S. 19 causes only a transitory reaction to the whey plate test which will recede to negative in 3 months. The method of performing this whey plate test is given by Cameron, Kendrick & Merriman(159). Whey is obtained by adding 2 drops of rennet to approximately 10 ml. of a composite sample of milk from each quarter and incubating overnight at room temperature or for 2 hr. at 37° C. The test is conducted in a way similar to that of the blood-serum plate test using 0·08, 0·04, 0·02 and 0·01 ml. of whey with one drop of antigen. It is read after 5–6 min. and the results recorded as titres 1, 2,

3 and 4, respectively. The results of blood serum and whey tests on 5062 animals showed 96% agreement between the two tests, but where there was disagreement the whey plate test appeared to be more efficient. Cameron (160) considers that animals showing titres to the whey plate test of 3 or 4 should be considered as infected unless they were vaccinated within the previous 3 days. Whey titres of 2 may be regarded as suspicious on the original test and positive if they persist on retesting. A comparison of the blood-serum and whey plate tests on 20,000 cows in California was made by Cameron (161). He is of the opinion that by all criteria the whey test is just as good for diagnosis as the blood-serum test. In contrast to the above papers from California all of which are in praise of the whey plate test, Reopke, Stiles, White & Driver (162) are of the opinion that it is unsuitable as an official diagnostic test under present conditions in Minnesota. Of 204 reactor animals, fifty-three were classified as negative by the whey plate test, and *Br. abortus* was isolated from the milk of ten of these fifty-three. In addition, marked variations were found between the titres of milk from different quarters of the same animal. Stiles, Roepke, Driver & Anderson (163) report further studies which strengthen this opinion. Tube-agglutination tests were done by Anczykowski (164) on forty-eight positive and seventy-one negative samples of cows milk and no difference was found between the results of whole milk and whey-tube-agglutination tests.

The capillary-tube test mentioned in a previous review of this series (165) has recently been reported by Jameson (166) to be useful as a screening test for quarter samples of milk submitted for culture of *Br. abortus*. In a series of 2959 samples the capillary-tube test detected all of 173 samples subsequently found to be positive by culture. The milk-ring test failed to detect three of the positive samples. Milunović (167) compared the milk plate test and the blood-serum-agglutination test and found that both tests agreed in 200 samples from infected herds. Comparison of the blood-serum and milk test were made by Feja (168) and he recommends that both tests be used for diagnosis.

Very few papers have been published in recent years on blood-serum-agglutination tests. Alessandro (169) found that there was a reduction in the agglutination titre of serum on incubation at 56° C. for 18 hr. compared with incubation at 37° C. for 24 hr. This phenomenon was observed in 94% of animals from non-infected premises after a single vaccination with S. 19, but only in 36% of naturally infected animals which had never been vaccinated. Erbin (170) describes a ring test in which coloured antigen, blood serum and milk are mixed. After allowing the mixture to stand for 5–10 min. it is centrifuged for 10 min. at 1000 r.p.m. This test is claimed to give a much clearer ring in positive cases and to avoid false positive reactions caused by fat globules or the presence of contaminating bacteria in the milk. Wiest (171) is of the opinion that the serum-ring test is inferior to the tube-agglutination and flocculation tests, and Brömel (172) considers that it is not a substitute for the rapid plate-agglutination test. Considerable variation was found by Ehrsam (173) in the agglutination titres of blood sera when fresh and after 24 hr. in cold storage. He recommends storage of all samples for 24 hr. Cedro, Cisale & Cacchione (174) describe a test with whole blood for diagnosis of brucellosis in the abattoir. The results of this test were corroborated by the serum-agglutination test. A modified slide agglutination method using dried brucella antigen stained *intra vitam* is given by Nørrung (175) in which 0·02 ml. droplets of 4% tetrazolium-stained brucella antigen are evaporated to dryness on cellophane. A droplet, with 0·05 ml. of saline and 0·01 ml. of serum, is stirred on the card-mounted cellophane, warmed if necessary and examined for agglutination. In a comparison with the tube-agglutination test Nørrung found that of 365 sera, 159 were negative and 206 were positive to both tests. A cup test for the rapid serological screening of guinea-pigs infected with *Br. abortus* is described by Jameson (176). This test, which proved slightly more sensitive than the tube-agglutination test, was applied to the sera of 7006 guinea-pigs 6 weeks after milk inoculation. Of 1034 sera positive to the cup test 86% of the guinea-pigs concerned proved to be infected on spleen culture. Surface-fixation tests following the technique of Castenada (177) reported in the previous review of this series (9) have been done by Caldas, Machado & Planet (178) with suspect sera and milk. Hunter & Colbert (179) give details of the preparation of a specific antigenic cholesterol-lecithin extract of smooth *Br. abortus* for use in the microscopic test and in the tube-flocculation test. Referring to

the complement-fixation test, Wiśniowski (180) found a solution of glucose 2·05 g., sodium citrate 0·8 g. and sodium chloride 0·42 g. in 100 ml. of distilled water very useful for the preservation of red blood cells. The guinea-pig serum used as complement is first tested for the presence of brucella antigens; it is then pooled and divided into 1·2 ml. lots and freeze-dried. In this state the complement can be stored for several months if protected from light. Adult cows (181) vaccinated with S. 19 gave positive reactions to both the complement-fixation test and the agglutination test within a few days of vaccination, but then the complement-fixation antibodies gradually disappeared and were absent at 10 months, while the agglutinins remain active for years. He suggests that repeated tests by both methods may differentiate vaccination titres and those following natural infection. Trilenko (182) found that the slow complement-fixation test in experimentally infected rabbits and cows became positive earlier than other tests, and that it could be used to test for brucella antigen in the stomach of the aborted foetus. The serum under test (183) is inactivated at 63° C. for 30 min. and the mixture of antigen, serum and complement is kept at 0–4° C. for 16–18 hr. and then at room temperature for 10–20 min. before the haemolytic system is added. In 2015 cattle in nineteen herds this test detected 526 reactors compared with 400 by the ordinary complement-fixation test and 240 by the agglutination test. These results, however, do not appear to have been confirmed by post-mortem examination.

The complement-fixation test is considered by Hajdu (184) to be more reliable than the agglutination test for the diagnosis of brucellosis in cattle because of the frequent occurrence of non-specific agglutinins. A preliminary trial of a test for detecting non-specific agglutinins has been made by Stiles, White & Roepke (185). The test is based on the use in the plate test of an acidified antigen which according to Hess (186) inhibits non-specific agglutinins, but the results were conflicting. Roepke, White, Stiles & Driver (187) report that most reactions of non-specific agglutinins are inhibited if the pH of the serum-antigen mixture of the 1 : 25 dilution of the serum plate test is adjusted to 4–3·5 by the addition of concentrated acetic or lactic acid to the plate test antigen. Rose & Roepke (188) are of the opinion that there are at least two types of non-specific agglutinins and that the possibility that there are more should be investigated. Amarasinghe (189) studied the adsorption of non-specific agglutinins by the rough antigen *Br. abortus* 9 R. This technique leaves the specific antigens free to combine with the *Br. abortus* antigen. Referring to non-specific agglutinins in the milk he considers that heating milk for 2 hr. at 65° C. inactivates them without interfering with the specific reaction of the milk to the milk-ring test.

Incomplete antibodies demonstrated by the Coombs antiglobulin technique were found by Cruickshank (190) in almost all guinea-pigs inoculated simultaneously with *Br. abortus*. They were detected between the 20th and 30th day, and reached a peak between the 50th and 70th day at which time the titre was four to eight times that of ordinary agglutinins. Černý & Menšík (191) found that incomplete antibodies were not demonstrable by the Coombs test in serum from calves of infected cows taken before the ingestion of colostrum, but were present in samples taken after ingestion of colostrum. Significant titres persisted for 2–5 months after birth. Gourdon, Quinchon, Guillo & de Labonnefon (192) consider the Coombs test a sensitive method which allows high specific agglutination titres to be obtained. It seems able to detect specific antibodies early, when the serum possesses only incomplete antibodies, and also later when the agglutination titre has dropped and the usual sero-agglutinins remain negative. The test, however, is long and difficult and not suitable as a routine diagnostic method. Schaal (193) reports that the Coombs test did not reveal the existence of incomplete antibody in samples of serum and whey from cows with brucellosis.

The vaginal mucus-agglutination test referred to in previous reviews of this series (165, 9) was claimed by Schmid (194) to be reliable and of value for detecting cows with uterine infection.

Papers published during the period under review show an increased interest in Europe in allergic tests for the diagnosis of brucellosis. Intradermal tests on cattle using a polysaccharide antigen have been made by Bürki, Margadant & Mosimann (195). Seven cattle known to be infected gave positive results and eleven uninfected cattle gave negative results except for one doubtful reaction. Bürki & Mosimann (196) demonstrated neither an agglutinin-stimulating nor

a neutralizing action in an allergen which they prepared. The antibody on which the allergen reacts has no relationship to the agglutinins. The reaction of the allergen may be demonstrated *in vivo* by the intradermal test or *in vitro* by the precipitation test. The desensitizing action of the allergen was slight at the dosage used. Bürki (197) reports that intradermal and precipitation tests were negative in guinea-pigs repeatedly infected with S. 19, but with virulent infection most animals were positive to both tests. Diernhofer (198) used a non-agglutinogenic, specific fraction of the *Br. abortus* endo-antigen for the intradermal test in cattle. The allergic reactions obtained were found to be group specific for *Br. abortus*. By prolonged boiling of brucella antigen in the presence of acetic acid and subsequent precipitation of the polypeptides and lipids, Diernhofer (199) obtained a polysaccharide which was a suitable hapten for allergic tests. It was non-agglutinogenic and caused no interference with serological tests. Experiments indicated that this polysaccharide, if combined with an appropriate medium, may be suitable for immunization without increasing the agglutination titre. Nižnánsky, Krčméry & Ofúkaný (200, 201) prepared brucella polysaccharides for skin tests by three methods. When one or other of these allergens was used in the intradermal test in combination with the milk-ring test, the accuracy of the intradermal test was stated to be good. A brucellin prepared by Buzna (202) had no antibody-producing effect and therefore did not influence the result of serological tests. Animals vaccinated with S. 19 all reacted to the intradermal test 6 months later, but only 65 % reacted to the serological tests. After tests on six heifers experimentally infected with *Br. abortus* and on three infected herds and two newly infected herds it was considered by Ottosen & Plum (203) that the intradermal test was of value in determining the spread of infection in newly infected herds, and that used along with the milk-ring test it was valuable in the routine supervision of brucellosis-free herds. Bühlmann (204) on the other hand, using 'Berna' (Swiss material) and 'Pebu' (Danish material) for intradermal tests in cattle vaccinated with S. 19 and in infected animals, found the allergic test to be inferior to the conventional serological and bacteriological tests for brucellosis. Musiïko (205) has studied 'brucella hydrolysate', a preparation obtained by acid hydrolysis of brucella organisms. Iodination of the brucella protein did not reduce its allergenic properties, but acetylation, deamination and treatment with formol did reduce them. He concluded from this that certain nitrogen groups, particularly amino groups, are responsible for the allergenic properties of 'brucella hydrolysate'. Bugeac, Cristescu, Lelutiu & Moraru (206) compared the intradermal test using 'brucella hydrolysate' with the agglutination and complement-fixation tests. They concluded that the combination of the three tests gave more accurate results and enabled a larger number of infected animals to be detected than any one test singly. Lokteva (207) tested 6758 animals with the allergic ophthalmic test for brucellosis. They included healthy animals, tuberculous controls, animals in isolation units, and animals in every stage of infection. The eye test appeared to be specific. It became positive simultaneously with the complement-fixation test and remained positive for longer. The reaction was not always clear-cut the first time, and it should be repeated in 3–6 days when the eye has become sensitized after the first test. It is stated by Bozhko (208) that the ophthalmic test with brucella antigen is simple and specific. It gives reactions even in animals that have ceased to give positive reactions to the agglutination and complement-fixation tests. As it fails sometimes in recent infections it should always be used in conjunction with serological tests. Evidence is presented by Stinebring, Flick & Pomales-Lebrón (209) to show that guinea-pigs, experimentally infected with *Br. abortus*, and which do not show a positive skin test on intradermal injection of bacterial extract, usually possess a 'tissue' sensitivity (*in vitro*) to this antigen. Tissue-culture studies on bacterial allergy in experimental brucellosis have also been made by Heilman, Howard & Carpenter (210). The study was concerned with the reaction in tissue culture of spleen from normal and brucella-infected guinea-pigs to different concentrations of a heat-killed suspension of *Br. suis*. The degree of toxicity was based on the inhibition of migration of wandering cells and upon the morphological appearance of stained sections of tissue cultures. The suspension was more toxic for infected than for normal spleen. The toxic effect was reduced by the presence of serum or plasma containing specific antibodies.

B. *Bacteriology*

Reference was made in the previous review of this series (9) to 'W. medium', a selective medium for the isolation of brucella organisms from contaminated materials. Carrère, Roux & Suire (211) refer to this medium and stress the difficulties of obtaining some of the constituents, especially actidione and circuline. They have evolved a medium using products more easily obtained. It consists of albimi agar to which are added sunoxol, eosin, nystalin, polymyxin and bacitracin. Baumgartner (212) found that for the demonstration of brucella in milk 'W. medium' was superior to the crystal-violet agar plate with added penicillin, because it inhibited the growth of extraneous organisms and gave a better growth of brucella. Tests on milk from thirty-one herds showed that although 'W. medium' may not reveal very low concentrations of brucella in mixed milk, it is a reliable medium for testing milk for human consumption. A medium containing polymyxin B sulphate, penicillin, actidione and gentian violet is described by Mair (213). This medium detected brucella in 80·7 % of samples of bulk milk from infected herds giving a positive milk-ring test as compared with 85·3 % by guinea-pig inoculation. Results can be obtained within a few days by Mair's method and even low levels of infection can be detected. Penicillin-susceptible strains were inhibited, however, and serious contamination sometimes occurred. Kozłowski (214) found a selective medium incorporating 3 % agar, 5 % horse serum, 1 % glucose, 1 : 200,000 gentian violet and 1 : 100,000 malachite green, very useful for primary isolation of brucella strains from contaminated material. The growth of fungi, which interfered with the cultural examination of milk samples for brucella, was overcome by Brodhage (215) by the addition of 300–400 units of mycostatin per ml. of milk. Morris (216) reports that brucella organisms grew well with 65 hr. of incubation on a selective medium containing 5-nitrofurfur-methylether, bacitracin, polymyxin and actidione, while organisms normally occurring in culture from faeces and soil were completely suppressed. A specific antiserum against a bacteriophage of brucella was prepared by Fomicheva, Akulova, Apollosov, Kushina & Kostyaeva (217), and added to culture media used for the diagnostic isolation of brucella from aborted foetuses. By this method cultures of *Br. melitensis* and *Br. abortus* were obtained from thirty-nine bovine and thirty-six ovine foetuses compared with sixteen and seventeen, respectively, for media without the antiserum. Studying the survival of *Br. abortus* in dung, King (218) found that from a broth culture of strain 2308 mixed with sterilized dung and placed near the bottom of a dung pit, brucella could not be cultured after a 4 hr. period.

Few papers referring to the typing of brucella species appear to have been published in the past 2 years. Schindler (219) reports that of fifty-five strains of brucella typed by determination of CO_2 requirements, fuchsin and thionin resistance and type specific agglutination, twenty-nine were typical *Br. abortus*, twelve were *Br. suis* and four were *Br. melitensis*. The remaining five *Br. abortus* and five *Br. melitensis* strains gave atypical reactions with one or other of the differential methods. Nižnánsky & Krčméry (220) found the determination of catalase and urease activity to be of value in differentiating between *Br. abortus* and *Br. suis*. They have also studied (221) the lipase and amylase activity on the three species and found that the activity of hydrolytic enzymes was four to five times higher in *Br. suis* than in the other two species. In this connexion Janok, Nižnánsky & Krčméry (222) have described a rapid electrometric method for determining the lipase activity of brucella. Godglück & Marggraff (223) have confirmed by two methods that *Br. suis* can be differentiated from the other two species by its superior production of urease. Richardson (224) has found that the quantitative determination of the cytochrome oxidase activity of brucella species permitted the differentiation of *Br. abortus* and *Br. suis*, but not of *Br. melitensis*. Parnas (225) claims to have transformed the biochemical and serological properties of the three species (he uses the term 'variants') by growing any one of them in a substrate prepared from one of the other two species. The substrate is made by growing the organisms and then destroying them by ultrasonic waves of 2800 kc./sec. for 90 min. followed by heating to 60° C. for 30 min. The live organism was given ten to twenty-five passages in this substrate which contained the metabolites of another species.

Microbiological analysis made by Chodkowski & Parnas (226) of strains of brucella isolated in

Poland has shown that they are peculiar varieties of intermediate type with low agglutinability and weak antigenic properties. Strains of *Br. abortus* sensitive to penicillin were studied by Sackmann (227). They were similar to S. 19 in their CO_2 requirements, and also in their thionin and thionin-blue sensitivity and catalase activity, but they were more virulent to guinea-pigs. Sacquet & Renoux (228) found in the course of immunization of a goat with living vaccine prepared from *Br. abortus*, strain 112, that it was possible to isolate from the vaginal secretions an atypical strain. Since the experimental conditions were strict, and exogenous infection impossible, it seems that an *in vivo* modification may have taken place. Kiris (229) claims that filterable forms of the three species of *Brucella* were sometimes obtained by passing broth cultures of the organisms through seitz filters. Were it not for the fact that he reports a variation in biochemical and serological properties after filtration, one would suspect the filtration technique.

Studies have been made by Sanders & Huddleson (230) on the influence of environmental conditions on the growth and dissociation of *Br. abortus*. They have shown that the atmosphere under which the cells of *Br. abortus* are grown in liquid medium is a factor as important as the metabolites in the causation and prevention of dissociation. Similarly, the conditions of growth, such as agitation, aeration or the fact that growth occurred in a shallow layer of medium are equally important. Blitek, Parnas & Zuber (231) found that the chicken embryo is very sensitive both to virulent and avirulent strains of *Br. abortus*. Schindler & Reusse (232) have found also that embryonated hens eggs are highly susceptible to infection with brucella. A single organism can initiate infection, and differences in virulence between various strains is manifested only by slight variations in time of death of the embryo.

Further work on the biochemistry of *Br. abortus* has been reported. Newton, Wilson & Wilson (233) have published a paper on the nucleotides and nucleic-acid synthesis by *Br. abortus*; Altenbern, Ginozo & Williams (234) on the roles of alanine and pantothenate in population changes in *Br. abortus*, and Altenbern, Williams, Kelsh & Mauzy (235) on the terminal oxidation and oxygen tension in population changes in this organism.

C. *Vaccination*

A numerical evaluation of the responses of vaccinated and unvaccinated cattle after exposure to virulent *Br. abortus* has been evolved by Goode, Manthei & Amerault (236). It has been called the 'index of infection'. If, after exposure, an animal aborts and *Br. abortus* is isolated from the genital tract a figure of 5 is allotted. If the animal calves at full time, but *Br. abortus* is isolated, a figure of 4 is allotted. An animal with an infection of the mammary gland is allotted the figure 3, and an animal showing a post-challenge rise in blood agglutinins of two or more dilutions is allotted the figure 2. The maximum figure that can be allotted is 10. By use of the 'index of infection' these authors were enabled to state that the immunity of cattle vaccinated subcutaneously as calves with S. 19 vaccine did not decrease as the calves got older. In an annotation on the degree and duration of immunity in cattle resulting from vaccination with S. 19, McDiarmid (237) points out that the use of the 'index of infection' makes it possible to compare American and British results if it is remembered that the American challenge dose is 733,000 organisms of *Br. abortus* strain 2308, and the British challenge doses 150×10^6 and 15×10^6 organisms of *Br. abortus* strain 544. He stresses that work in both countries shows that the immunity resulting from vaccination with 5·0 ml. of S. 19 in calfhood does not deteriorate with age.

Studies on the relative immunity to brucellosis of calves vaccinated intradermally compared with the immunity of those vaccinated subcutaneously with S. 19 vaccine have been reported in the last three reviews of this series (165, 82, 9). Further work on this subject has been done in recent years by Gilman & Hughes (238) using thirty calves, which were later challenged conjunctivally when 4 months pregnant. The approximate abortion rates were 7% for those vaccinated subcutaneously with 5·0 ml. of S. 19, 33% for those vaccinated intradermally with 0·2 ml. and 75% for the unvaccinated control animals. They point out, however, that with the decreased incidence of brucellosis that now exists, the smaller intradermal dose may give sufficient pro-

tection to natural infection. For use in South Africa, van Drimmelen (239) recommends the subcutaneous administration of *Br. abortus* vaccine as being easier for unqualified assistants. Field tests showed that the concentration of the dose (60×10^9 viable organisms) into 1 ml. instead of 5 ml. produced abscesses and lasting swellings. The successful use of S. 19 vaccine in Bulgaria in the control of an outbreak of brucellosis on a state-controlled farm is reported by Angeloff (240). Calves were given a subcutaneous injection of 5 ml. and cows 6 ml., of a vaccine containing 10×10^9 organisms per ml. Intradermal injection into the vulva of 50×10^9 organisms in 0·5 ml. diluent was also adopted and is believed to be more convenient and to produce a better and a more rapid immunity. In assessing the results of an experiment on vaccination and revaccination, Sieiro (241) divided his animals into immune and infected. Infected animals were further divided into 'abortion resistant' and those which aborted. All animals were challenged when 4–7 months pregnant. The results were as follows: of calves vaccinated with S. 19 at 10 months old, 57% were immune and 43% aborted; of calves revaccinated when 18 months old 75% were immune and 25% infected, but none aborted; of calves vaccinated at 18 months old 82% were immune and 18% infected, but none aborted, and of the unvaccinated controls 28% were immune, 72% infected and half of the latter (36%) aborted. Immune animals were those which calved normally and did not acquire an infection traceable by bacteriological or serological methods. Infected animals were those which aborted or from which virulent organisms were isolated or which gave a rise in agglutination titre.

The results of tests on blood and milk and the examination of placentas and post-mortem findings after immunization with S. 19 are given in a series of four papers by Seelemann, Börger & Meyer (242). The animals studied were those with a persistent titre to the slow serum agglutination test in dairy herds which, according to the flocculation test and milk tests, had long been freed from brucellosis. The percentage of reactors was higher amongst animals vaccinated at 10–15 months old than those vaccinated at 4–9 months old. Six of these persistent reactors were slaughtered and cultures and guinea-pig inoculations made from their organs and lymphatic nodes, but brucella could not be recovered. Agglutination titres, however, developed in guinea-pigs inoculated with material from one of the animals slaughtered 13½ months after vaccination. Seelemann and his colleagues failed to recover brucella from three calves slaughtered 2 days, 3 weeks and 7½ months after virulent infection. They recovered brucella, however, from three out of four calves slaughtered 5 days to 3 weeks after vaccination with S. 19, but not from five calves 6 weeks to 3½ months after vaccination. The serum of 110 calves vaccinated with S. 19 when 6–12 months old was examined periodically for agglutinins by Fontanelli (243). It was seen that about 1 year after vaccination the majority of animals gave negative reactions, and after 18 months all were negative. The white blood cells and serum proteins of cows following vaccination with S. 19 have been studied by Boda, Roško & Tomáš (244). The reaction of the leucocytes in the blood was most marked on the seventh day after vaccination before the maximum agglutination titre was reached. There was an increase in the number of lymphocytes and eosinophils, and the number of neutrophils appeared relatively reduced. The ratio of the cells gradually reverted to normal. The highest relative increase of γ-globulins was during the second and third weeks when the agglutination titre was at its peak. Greatorex (245) also has studied the blood picture of calves following vaccination with S. 19. Ninety-one clinically healthy calves of different breeds were studied and of this number forty-five were vaccinated and forty-six were left as controls. The animals were divided into four groups according to age, 4, 5, 6 and 7 months old. He found a rise in the coagulation time corresponding with the initial rise in the agglutination titres of the vaccinated animals. The erythrocyte count, packed cell volume and haemoglobin content of the vaccinated calves showed significantly reduced values by the fifth day following vaccination. A gradual increase in the total leucocyte count occurred as the agglutinins appeared, and this increase was due mainly to an increase in the number of circulating neutrophils. There was a reduction in the percentage of lymphocytes. Dale & English (246) found that vaccination with S. 19 of beef and dairy calves produced marked systemic responses. The rectal temperature was elevated by 3–4° F. for 2–3 days and did not return to normal till 5 days after

vaccination. Food consumption was 30% down on the third day and did not return to normal till about a week after vaccination. Decowski & Żórawski (247) examined the milk of eighty-five cows before vaccination with S. 19 and daily for 14 days after it. The organism was isolated from the milk of one 9-year-old cow in poor bodily condition, 24 hr. after vaccination.

An account of the technique and apparatus for the production of S. 19 vaccine at Onderstepoort is given by van Drimmelen (248, 249). Shake flask cultures are employed and the medium prepared contains peptone, glucose phosphate and yeast extract. This method has certain advantages over the use of potato-infusion agar under South African conditions. Concentrated freeze-dried vaccine containing 60×10^9 viable organisms per dose is prepared by sedimention of the organisms by means of 0·05% sodium carboxymethyl cellulose. This substance has the advantage also of increasing the survival rate after freeze-drying and during storage. A method of improving the survival rate during the lyophilization of S. 19 is described by van der Scheer (250). A suspending medium containing 2% tryptose, 0·5% neutralized ascorbic acid and 0·75% thiourea is used. In 100 separate batches of vaccine dried in this medium the average survival rate after primary drying was 88% and in the finished sealed ampoules 82%. A viability protective factor that enhances the survival of *Br. abortus* during freeze-drying is reported by Bergmann, Halleck, Mechalas & Tenney (251). The factor is extracted from the supernatant fluid of aged cultures and also from young and aged cells. It was purified to a waxy material by solvent extraction, and its chemical and enzymic characteristics as well as its spectroscopic characteristics have led to the assignment of a lecithin-like structure to the active component. Van Drimmelen (252) has developed a method of testing S. 19 vaccine for viability by means of a reaction which depends on the enzyme activity of the organisms present. A small quantity of vaccine, ready for use, is diluted with saline and added to sterile powdered tetrazolium in a small ampoule and the mixture held at body temperature for 1–2 hr. Fresh vaccine and fully-viable stored vaccine show a deep-red colour, but there is no reaction with vaccine of low viability. Seelemann (253) emphasizes the need for standardization of brucella vaccines in Germany. In his experience the injection of 5·0 ml. of S. 19 containing 2^9–3^9 organisms per ml., repeated after an interval of 4 weeks is a sound method of calfhood vaccination. King, Edgington & Frank (254) have used Huddleson's *Brucella* M vaccine under field conditions, and found a marked reduction of the abortion rate of vaccinated cattle in comparison with the control animals (9 and 27%, respectively). Schoenaers & Kaeckenbeeck (255) have confirmed the results of previous experiments in which the vaccination of cattle with *Brucella* M vaccine gave a satisfactory protection against *Br. abortus* infection.

Trials using a vaccine prepared from *Br. abortus* strain 68 are reported. Tuzova (256) states that this vaccine proved safe and effective; the animals, however, developed agglutination titres of 1:200–1:3200 which persisted for 9–12 months. The complement-fixation test and allergic tests were negative. Yuskovets & Tuzova (257) claim that inoculation of 534 cows with this vaccine protected 98% of them from brucellosis, but the method of exposure of the animals is not given in the original abstract. Twenty-five batches of aluminium hydroxide vaccine were prepared from *Br. abortus* strain 68 by Yuskovets & Kolesova (258). After tests on guinea-pigs, mice and sheep, 4145 cattle on thirteen infected farms in various parts of the U.S.S.R. were vaccinated, and the results showed that abortions ceased or were greatly reduced. A study of vaccinal immunity to brucellosis in farm animals by a cytological method has been made by Borzenkov (259). He claims that the establishment of immunity in an animal is accompanied by active division of the cell nuclei in the exudate from the site of injection of the vaccine, and that therefore by studying the macrophage reaction in stained smears of the exudate it is possible to estimate the immunogenic properties of the vaccine.

In addition to work on live vaccines, some experiments using dead vaccines have been reported in the period under review. An extension of the work of McEwen & Samuel, which was reported in the previous review of this series (9) in which a comparison of the immunity produced in guinea-pigs by inoculation of living, heat-killed and adjuvant vaccines prepared from *Br. abortus* strain 45/20 and S. 19, has been made by McDiarmid & Sutherland (260). They confirmed that a killed non-agglutinogenic adjuvant vaccine prepared from the rough strain 45/20 (McEwen)

Br. abortus is capable of engendering considerable immunity in guinea-pigs in no way different from that obtained with the living strain, and it is considered that this new antigen is well worthy of further trial as an immunizing agent against brucellosis in adult cattle. Extended trials of this new vaccine in cattle of breeding age have begun at Compton. Frahm & Lembke (261) prepared a vaccine from acetone-killed *Br. abortus* adsorbed on aluminium hydroxide. Following tests on mice it was injected into 500 cattle on infected farms, two injections being given 4 weeks apart. Vaccination was assessed as successful solely on the basis of absence of abortion, absence of brucella in the placenta and an increase in the γ-globulin in the blood. Challenge experiments were not done. The same authors (262) compared the killed-vaccine with S. 19, and were of the opinion that it was of value because agglutination titres were lower than with S. 19. Figures for the incidence of abortion in each group are not given. The brucella vaccine prepared at the Ukrainian Institute for Experimental Veterinary Science (263, 264) is a dense suspension of agar cultures of virulent *Br. abortus* inactivated with potassium alum and crystal violet. In field trials on ten infected farms during 1950 to 1953 over 5000 cattle were vaccinated. Two intramuscular injections each of 5 or 10 ml. were given with an interval of 15–20 days. The animals were revaccinated after 12 months and where necessary annually thereafter. Blood serum taken 3 weeks to 2 years after vaccination protected mice from infection with 2–3 LD_{50} of brucella. Vaccination protected 91–99% of the cattle from infection by contact. Tronin (265) reports that since 1948 the progress towards freedom from brucellosis has been very rapid in the Vologrod region where a formal-killed vaccine has been used on a large scale.

It is reported by Hollister (266) that in a large herd, cows vaccinated against brucellosis between the ages of 6 and 8 months were given a mixed vaccine of pasteurella, a corynebacterium, streptococci and staphylococci. The herd had been declared free from brucellosis 6 weeks before, but 22 days after vaccination 21% of the animals gave positive reactions to the serum-agglutination test for brucellosis, and 14% doubtful reactions. Attempts to reproduce this phenomenon under controlled conditions were unsatisfactory. It is suggested that because of the risk of such anamnestic reactions vaccinated cattle should not be tested for brucellosis for at least 60 days after injection of serum or vaccine. Berman (267) also reports that certain commercial vaccines against *Pasteurella septica* stimulate the production of agglutinins in cattle which have been vaccinated with S. 19.

Studies on the extraction of brucella antigens have been made by Foster (268). He points out the possibility that alterations in molecular structure are caused by the usual methods of extraction and preservation, and that these may affect the biological activity of the antigen. A multivariable system of plasma fractionation which he uses should minimize this risk.

D. *General*

Some figures have been published recently giving the estimated incidence of brucellosis in various parts of the world. Brill & Gołebiowski (269) describe investigations in Poland and state that serological tests confirmed brucellosis infection in 66–72% of the adult cattle, pigs and dogs which were examined. The infection rates in man and horses were 17 and 21%, respectively. Kralj, Brudnjak, Rižnar & Hajsig (270) report that 0·66% of 1731 cows and heifers in the Zagreb district of Yugoslavia gave a positive reaction to the milk-ring test. Landi (271) reports that between the years 1951–55 the annual number of outbreaks of bovine brucellosis in Cremona rose from 29–66. It was found by Schaal (272) that 20% of 1600 cows examined at Duisburg abattoir yielded positive agglutination titres of 1:50 or higher, and that 12% yielded titres of 1:25. Investigation into the presence of brucella in the milk of 1696 cows in the province of Milan has been made by Maestrone (273). Blood agglutinins were found in 65% and agglutinins in the milk of 6·2% of these cows. Almost three-quarters of the 6·2% shed brucella in the milk. Over the past 3 years 2078 animals on ninety-three farms in Kenya were tested by the milk-ring test by Thorold & Holmes (274). They found that 20% of the animals gave a positive reaction revealing 33% of the farms to be infected. In a survey of bovine brucellosis in French West Africa reported by Perreau (275) nearly 2000 agglutination tests and 1000 milk-ring tests were made. The average

incidence was 12%, the Lake Chad region having the highest incidence (24%). There was great variation in the incidence in contiguous villages. Tests done on milk from dairies in the Provinces of Buenos Aires and Cordoba by Morán & Maubecín (276) showed that the milks from 96% of 1345 dairies in the former province and from 65% of 1227 dairies in the latter province were positive to the milk-ring test.

A study of the role of ticks in the spread of brucellosis has been made by Gudoshnik (277). The experiments were done in western Siberia in several old active foci of brucellosis and also on farms from which the disease had been eliminated. *Dermacentor marginatus* and *D. pictus* were the two species of ticks collected from animals and pastures. *Brucella* could not be cultured from the ticks, but when the ticks were allowed to feed in batches of ten to fifty on guinea-pigs, they transmitted *Br. melitensis* to some of the guinea-pigs. In another experiment, ticks allowed to feed on infected guinea-pigs spread *Br. melitensis* to a healthy ram. The ram gave a positive-agglutination test 3 months later and the organism was isolated from it. Sivovski & Khristoforov (278) report that colostrum from thirteen cows infected with brucellosis had high 'serological' titres and frequently contained organisms. The calves receiving the colostrum became infected within a few days and demonstrated positive serological tests. The authors concluded that in cows with established brucellosis no antibodies are supplied to the embryo.

Some papers on the treatment of brucellosis have been published during the period under review, and probably the most important of these refers to the treatment of undulent fever. Gost (279) reports the successful treatment of twenty patients, each giving a positive blood culture of *Br. melitensis*, with a new antibiotic 'Cathomycin' (novobiocin). Kalinin (280) recommends the use of Pentoxyl (5-oxymethyl-4-methyluracil), which is a substance that stimulates leucopoiesis. In his method of treatment he combines Pentoxyl with biomycin, levomycetin or syntomycin. In the treatment of guinea-pigs experimentally infected with *Br. melitensis* or *Br. abortus*, Anina-Radchenko & Bretslavetz (281) claim some success with 'Tegan' (composition not stated) or phthivaze (an iso-nicotinic acid derivative). Bosio (282) claims that a combination of penicillin and dihydrostreptomycin in doses of 2 mega units and 2 g. respectively, for 5 days gave clinical cures in eight cows with purulent endometritis due to *Br. abortus*. There must be some doubt in this case whether the endometritis was in fact caused by this organism. Yuskovets (283) concludes from experiments on mice and guinea-pigs and from some trials in cattle that it is possible with a hyperimmune serum to control the spread of infection of brucellosis and to ensure a maximum of normal calving in infected herds. Treatment of infected cows by repeated vaccination with S. 19 has been attempted by Rotov (284). A series of papers referring to the substance menadione has been published in the journal *Zooprofilassi*, and three of them, by Trivellato (285), Fabiani & Grazzini (286) and Giacomini (287), refer to the treatment of brucellosis with this substance. It is not clear why menadione should be expected to be of value in the treatment of brucellosis.

Interest in the effect of bovine brucellosis on man appears to be increasing. Cruickshank (288) has tested all four strains of *Br. melitensis* which have been isolated in Britain and found that they behave in guinea-pigs in a similar way to virulent strains, bacteraemia persisting for 4 weeks or longer. This finding does not support the suggestion that these strains are of low virulence. Wallis (289) describes ten cases of brucellosis in children in Britain. He points out that the disease is common in country districts, but is often missed. It may also be seen in cities early in the autumn after children have returned from holiday in the country where they have drunk raw milk. Apparently a fallacious diagnosis of psychoneurosis is often made. Joubert & Grebot (290) suggest applying the ring-test to churn milk as a method of controlling the danger of brucellosis to the human population. The incidence of *Br. abortus* agglutinins was assessed by Lawy & Hampson (291) in 8209 human sera, which were submitted for the Wassermann test in the Lincolnshire area of England from 1948 to 1953 using Huddleson's slide agglutination test. The incidence of sera with a titre of 1:80 or more declined from 1·05% during 1948–50 to 0·64% during 1951–53. Although barely of statistical significance this decline appeared to be associated with a decrease in the number of producer retailers, an increase in the heat treatment of milk and

an increase in the number of vaccinated animals. Nucciotti (292) states that the number of cases of brucellosis in Cremona, where the population is 378,000, rose from 2·7 to 49·0 per 100,000 inhabitants during the period 1941–54. In a few cases where bacteriological studies were made, *Br. melitensis* was responsible for the infection. According to Bławat (293) 20 out of 108 auxiliary veterinary personnel examined in Poland during 1952–53 had symptoms of systemic brucellosis. Following examination by serological and allergic tests thirty-one were considered to be infected. Infection in a human subject with S. 19 diagnosed serologically and bacteriologically is reported by Ertürk (294). Summa & Bethcke (295) report that five employees of an abattoir developed brucellosis shortly after slaughter of 400 infected sheep. Burgisser (296) reports the isolation of *Br. intermedia* from the milk of a cow and a goat in Switzerland which were a source of infection for human beings, and Aleandri & Gargani (297) found what appeared to be an intermediate strain of brucella infecting fifteen farm personnel in Florence. Zdrodowski, Vershilova & Kotlarova (298) give an account of large-scale immunization of humans in the U.S.S.R. against *Br. melitensis* using live cultures of either *Br. abortus* 19-B/A (an American strain) or *Br. abortus* M (a Russian strain). The morbidity rate was reduced by three to eleven times after immunization of various classes of people living in endemic areas compared with comparable non-vaccinated inhabitants.

Papers referring to various aspects of brucellosis that do not come under any of the special headings used in this article are reviewed here. Holland & Picket (299) have studied the intracellular behaviour of brucella variants in chick embryo cells in tissue culture. They found that smooth brucellae were ingested by fibroblasts and multiplied within them in tissue culture, whereas non-smooth variants and S. 19 neither multiplied nor survived long unless the cells were massively infected. Brucellae remained viable intracellularly for over 30 days despite the presence of a bactericidal concentration of streptomycin in the tissue-culture medium. This observation would appear to have a bearing on the treatment of brucellosis. Pomales-Lebrón & Stinebring (300) observed intracellular multiplication of *Br. abortus* in normal guinea-pig mononuclear phagocytes, but multiplication was significantly restricted in mononuclear phagocyte systems that are *Br. abortus* immune. Because *Br. abortus* was isolated from a carpal hygroma in 1954 Coid & Vaughan (301) compared the incidence of this condition in infected and non-infected cattle in February 1954 and February 1955. They found that the incidence of carpal hygromas in cattle infected experimentally with *Br. abortus* was not significantly higher than in the control animals. Changes in the rat placenta and foetus following experimental infection with *Br. abortus* have been studied by Payne (302). He concludes that *Br. abortus* is capable of causing lesions in the placenta of pregnant rats associated with vascular derangements and invasion of polymorphs. The foetus may die or become subnormal in weight and may fail to accumulate glycogen before birth. Chernysheva (303) studying the role of the reticulo-endothial system (R.E.S.) in immunity to brucellosis found that when S. 19 is injected the reaction of the R.E.S. is stronger and more rapid than after the injection of a virulent strain.

E. *Control and eradication*

The progress made recently in control and eradication programmes that are being carried out in various parts of the world will now be referred to. The present status of the State-Federal programme for the control and eradication of bovine brucellosis in the U.S.A. is discussed by Ranney (304). This programme began in 1935, but most progress has been made in the last 10 years. In 1935, 11·5% of the 3·3 million cattle tested were reactors, whereas in 1956 only 2·2% of the 16·7 million cattle tested were reactors. By December 1956, 19% of the counties in the U.S.A. were certified as free from brucellosis, and in 16% of them area control work was in progress, but it appears that in the remaining 65% no area control work was being done. One of the greatest assets to the programme was the adoption in 1952 of the milk-ring test as an official part of the programme. Progress of brucellosis eradication under range conditions in Montana is discussed by Wilbur (305). The present incidence of reactors is 1% as against 4% in 1952. The eradication campaign is on the following lines: (1) limitation of the breeding season to 60 days;

(2) the culling of dry cows in the autumn; (3) the testing of breeding cattle in the autumn 4 months after calving followed by the disposal of reactors with a titre of more than 1:50, the remaining animals being retested at least once; and (4) vaccination of heifer calves. Bryan & Graham (306) give details of a brucellosis-eradication programme in twelve Illinois state-owned dairy herds, where the herds are maintained as closed units. Animals classified as reactors to the blood agglutination test were slaughtered, but suspicious and negative reactors were retained, and vaccination was discontinued. In 1950, of 1609 animals 69·2% were negative and 6·1% positive to the agglutination test, the remainder giving doubtful reactions, whereas in 1956 of 1670 animals 98·6% were negative. Information on the history of control plans in Missouri has been published by Price (307).

Turning now to Europe, the control of bovine brucellosis in Denmark is described by Thomsen (308) and Suurballe & Nielsen (309). A large part of Denmark has been freed from brucellosis and the use of vaccine has almost ceased. Eradication is based on the registration of free herds after repeated milk-ring and serum-agglutination tests. Vaccination is not allowed at any time during the test period. After registration the milk is tested regularly and when necessary the blood serum is also tested. Only registered animals are allowed in the stock markets. Tilgner, Dehning & Neumann (310) report on the campaign against *Br. abortus* in Schleswig-Holstein, where the slow-agglutination test is used. Milk from individual cows is tested four times a year and churn milk at less frequent intervals. A report of a commission on bovine brucellosis in the Netherlands (311) states that the milk-ring test was done on the milk of 2,752,000 cows in 29,000 herds during 1955. The tests showed that 63% of the herds were free from brucellosis. The scheme for control is based on the regular examination of herds by the milk-ring test supplemented by blood-serum-agglutination tests. The use of vaccine is restricted. Cows reacting to diagnostic tests are slaughtered when convenient, but compulsory slaughter with compensation is not envisaged at the present stage of eradication. Hess (312) states that in the Canton of Zürich control of brucellosis is based on the slaughter of cows excreting the organism in the milk or from the uterus. Detection of excretion in the milk is based on the milk-ring test and of excretion from the uterus by bacteriological test on material from aborting cows. Subject to certain conditions the owners of slaughtered cows are compensated up to 80% of the market value of the cow. Lipnicki (313) reports that the control of brucellosis in Poland is based on vaccination with S. 19 and elimination of infected animals. Over 200,000 animals have been vaccinated since 1948, and the results have been satisfactory since abortions caused by *Br. abortus* are now very rare.

The method which is being used for the eradication of brucellosis in the U.S.S.R. is given by Yuskovets (314). Control measures enforced by the state are based on the segregation of infected animals and on the sanitation and quarantine of infected herds. Infected herds are subject to monthly tests and are declared clear only after two successive negative tests and freedom from brucella abortion for a period of 6 months. Calves born to cows from infected herds are separated from those born to non-infected cows. Vaccination with an attenuated strain such as S. 19 is practised where complete segregation is not possible and where the incidence is high. The tube-agglutination and complement-fixation tests are the diagnostic methods used. Two papers (315, 316) also from the U.S.S.R. refer to cattle kept on isolation farms. It is claimed that after 3–5 years with periodic culling of aged and aborting cows and provided that no new infection was introduced, the animals became negative to diagnostic tests.

A note of warning must be given with regard to eradication and that is that care must be taken not to allow infection to enter a herd which has been long free from infection, because Goode, Manthei & Amerault (317) have found that cattle raised in an experimentally brucella-free environment were more susceptible to experimental infection with virulent *Br. abortus*, than were susceptible cattle purchased from herds showing no evidence of brucellosis. Turning lastly to Britain where there are no compulsory schemes for the control and eradication of brucellosis, McDiarmid (237) states that clinical brucellosis has now almost disappeared and the well-known abortion storms are no longer a hazard. It is unfortunate, however, that the occurrence of

brucella in milk is still high. At present in Britain only one in every three heifer calves under 1 year old is receiving vaccine. Despite this, S. 19 has undoubtedly done more to reduce clinical brucellosis to negligible proportions than any other factor. It would do still more if a compulsory vaccination programme was introduced as another step towards eventual eradication.

III. VIBRIOSIS

A. *Introduction*

The importance of *Vibrio fetus* infection as a cause of infertility in cattle has been increasingly appreciated in recent years. In a paper by Pickard quoted by Hodges (4) the estimated losses from vibriosis in the United States are given as 137 million dollars per annum, a loss second only to that caused by mastitis. A recent publication by the Food and Agriculture Organization of the United Nations edited by Laing (318) gives details of the present state of knowledge regarding this disease. Reference is made to this publication several times in the sections that follow.

B. *Bacteriology*

Vibrio fetus is defined (318) as a Gram-negative vibrio found in material from naturally infected animals and is seen in fresh cultures in short S-shaped forms, and less often in slightly more elongated forms. Very long spiral forms are often found in old cultures. It is usually motile. Most strains are micro-aerophilic and grow best in an atmosphere containing 10–20% CO_2, although some strains will grow aerobically when adapted to laboratory conditions, and Reich, Morse & Wilson (319) have found that CO_2 at a concentration greater than that usually present in air is not essential for growth of *V. fetus*. They found that forty-two strains they tested responded with considerably better growth to a micro-aerophilic environment containing 6% oxygen. The concentration of oxygen was lowered by using helium or nitrogen as a diluent. Many strains were apparently sensitive to hydrogen and gave increased yields in its presence. Kiggins & Plastridge (320) state that the optimum gaseous environment for growth on blood agar is 5% oxygen and 10% CO_2, either under partial vacuum or adjusted to 96% atmospheric pressure by adding hydrogen or nitrogen. Voigt (321) recommends culture of samples of genital secretions for 3 days in liver broth in an atmosphere containing 10–15% CO_2 at a pressure of 20 mm. Hg.

Differential culture tests. *V. fetus* is recognized as (318) being catalase-positive with a catalase number of thirty or more, it reduces nitrates to nitrites, does not produce H_2S and grows only on or near the surface of deep stab cultures in semi-solid thiol medium. Such organisms have been isolated from aborted foetuses and from the male and female reproductive tracts of infertile animals. Organisms of similar morphology and general cultural characters, but which are catalase-negative, reduce nitrates to nitrites, produce large quantities of H_2S and grow well in deep stab cultures of semi-solid thiol, may be found either with the catalase-positive type, or alone in the reproductive tracts of infertile animals, but they are also present in fertile cows and bulls, and are not thought to be associated with infertility. They have not been isolated from aborted foetuses and are not considered to be *V. fetus*. One strain, morphologically like *V. fetus* and found in semen, in the vagina, and frequently in the foetal membranes, but which is a strict anaerobe and produces large quantities of H_2S, has been designated as *V. bubulus*. After testing sixty-six cultures of vibrios of disease origins, Kuzdas & Morse (322) question the advisability of identifying isolates from bovine and ovine urogenital tracts as *V. fetus* on the basis of the catalase test alone, since vibrios from soil water and cheese were catalase-positive. They would define *V. fetus* as catalase-positive, but not producing H_2S and unable to grow on initial isolation in a medium containing 3·5% sodium chloride. Various recently published papers tend to confirm these characteristics of the vibrios found in the bovine genitalia. Chevé & Gauthier (323) found that all seven strains of *V. bubulus* tested, and also two of fifty-one strains of *V. fetus*, produced H_2S. Mundt (324) studied the properties of seventy-six strains of *V. fetus*, and fifty-six strains of *V. jejuni*, a vibrio from the intestine of cows. Frank, Bryner & Caruthers (325) frequently isolated

catalase-negative vibrios from unbred bulls, but not from virgin heifers. Catalase-positive *V. fetus* was not isolated from unbred bulls and virgin heifers. The necessity for isolation and culture to distinguish non-pathogenic strains from *V. fetus* is stressed by Lindqvist (326). He isolated seven strains of vibrio from semen and cervical mucus which were catalase-negative, produced H_2S, grew in deep agar stabs and were non-infective to maiden heifers. Cultural examination of semen and preputial washings of fifty-two bulls was made by Morgan, Melrose & Stewart (327). *V. fetus* was not isolated direct from the bulls, but it was isolated from the genital tract of virgin heifers test-mated to sixteen of the bulls. Twenty-six strains of catalase-negative vibrios were, however, isolated direct from the bulls. Hubrig (328) describes the cultural characters of fifty-seven strains of vibrio isolated from foetuses, preputial washings and bull semen. He identifies all strains as *V. fetus*, but suggests a subdivision into catalase-positive strains from foetuses and catalase-negative strains from bull semen and preputial washings. King (329) gives the cultural and biochemical properties as well as the serological relationships of ten animal and seven human strains of *V. fetus*, and of three animal strains of *V. bubulus* and four human strains of a related vibrio.

Colonial variations. Considerable variation in colonial type occurs in cultures of *V. fetus* on solid medium (318), but what might be considered the basic types are shiny, pale-grey, semi-translucent, flattened conical colonies, small and large ones often appearing on the same plate. Sometimes the growth appears as if several colonies had coalesced into a large mucoid mass, but at other times the only evidence of growth is a slight 'frosting' of the surface of the medium. Bond (330) describes three main types of colony, corresponding to the smooth, rough and mucoid variants as described in other groups of bacteria. They were easily distinguished when stained with crystal violet and observed by oblique transmitted light. He gives the 'frosted glass' type of colony as a possible fourth variant, but states that so far it has not been isolated in pure culture. The smooth forms of the strains he selected for examination were catalase-positive; they did not produce H_2S and did not grow in deep stab cultures, whereas the mucoid variants of some strains were catalase-negative produced H_2S and grew in deep stab cultures. Examination of stock strains of *V. fetus* from bovine, ovine and human sources, by Ristic, Herzberg & Sanders (331) showed that most cultures contained a high percentage of non-smooth variant forms. The cultures had been maintained in semi-solid albimi broth and transferred monthly using large inocula which would have favoured selection of mutant types if any sort of selective differentiation between variants existed. They describe five types of colony: smooth, rough, cut glass, mucoid and very rough. On the basis of the colonial and cellular characteristics of the parent culture and on the dissociation pattern of successive generations, Ristic, White & Doty (332) claim that saprophytic vibrio strains of bovine origin could be distinguished from *V. fetus*. Using blood-agar medium containing 20% defibrinated horse blood for the isolation of *V. fetus* from bovine material, Hubrig & Röhr (333) state that cultures of pathogenic strains incubated for 3 days in an atmosphere containing about 20% CO_2 formed bluish-white colonies with a metallic sheen, whereas colonies of non-pathogenic vibrios were yellow to yellowish-green.

Culture media. Details of the preparation of media used for the isolation of *V. fetus* and for the growth of antigen are given in the recent F.A.O. publication (318).

Kuzdas & Morse (334) have devised a selective medium for the isolation of *V. fetus* and related vibrios which will inhibit most of the troublesome bacterial contaminants. Unfortunately, species of the genera *Pseudomonas* and *Proteus* are not inhibited. The medium consists of albimi brucella broth 28 g., agar agar 5 g., ox bile (fresh) 40 ml., ethyl violet to give a final concentration of 1 : 800,000 and distilled water 1000 ml. The medium is autoclaved at 121° C. for 20 min. and then bacitracin 25,000 units, polymyxin B sulphate 5000 units and actidione 100 mg. are added to it aseptically. A method is presented by Reich, Dunne, Bortree & Hokanson (335) to grow *V. fetus* rapidly in large numbers in broth. This consists of aerating the broth with a gas mixture of 5% CO_2, 35% air, 10% hydrogen and 50% helium in a closed system. This procedure eliminates the need for agar in the medium, increases the total cell yield and halves the length of the lag phase. Robertstad & Morrison (336) describe a shaker technique which has been successfully

applied to the propagation of *V. fetus* for the production of a high yield of cells in a short period. With this technique the original morphology of a freshly isolated catalase-positive, gram-negative, short curved rod that did not produce H_2S remained constant through a 32 hr. incubation period. Evidence is presented by Trueblood & Tucker (337) which indicates that the addition of amniotic fluid to brucella agar in a concentration of 5% enhances the growth of *V. fetus*. Mundt (338) has used a nitro-cellulose membrane filter with a pore diameter of 200 mμ for the preparation of *V. fetus* antigen. Two drops of culture evenly spread over the surface of the filter and incubated for 3 days at 37° C. with CO_2 resulted in abundant growth. The antigen was obtained by removing the membrane and washing it with saline. This method obviates the need for repeated washing and purification of the organisms, processes that are necessary with liquid and solid culture media.

C. *Diagnosis*

The F.A.O. publication (318) to which reference has already been made, gives a short note on the symptoms of vibriosis. In the bull, no clinical abnormalities can be observed in animals which are carriers or transmitters of *V. fetus*, and the semen appears normal. In the cow and heifer, infection is at first acute and gradually assumes a chronic form. Infertility is usually associated with the acute form, and abortion with the chronic form, but abortion may also occur in the period of acute infection. At the onset of the disease, vaginal examination often shows a catarrhal vaginitis resulting in increased mucus secretion which may last for 3–4 months. Cervicitis and endometritis may develop. During the acute stage of the infection fertility varies, about 15–45% of animals becoming pregnant to the first mating, the remainder returning to service, often repeatedly. Usually the interservice periods are irregular and abnormally long. If mating is continued, most animals become pregnant after about 3 months. Later, the disease now being chronic, fertility becomes fairly satisfactory and it is only maiden heifers and cows not previously exposed to infection that become infertile. Some pregnant animals may abort, but the abortion rate is usually low. Abortions are most common about the fifth and sixth month of pregnancy. Accurate diagnosis of vibriosis can be made only by isolating the organism from material from suspected animals or by means of specific serological tests, but these latter tests are applicable only to females.

Much of the work on bacteriological diagnosis published during the period under review refers to infection in the bull. Florent (339) reports that in an agar containing brilliant green at a dilution of 1:40,000, *V. fetus* was isolated from 80% of samples taken from eighteen infected bulls, and Florent & Vandeplassche (340) are of the opinion that the use of a selective medium containing brilliant green is preferable to test-mating, since it provides such a quick and cheap method of diagnosis. Hubrig, Wohanka & Gränz (341) state, however, that the presence of *Proteus* and *Pseudomonas* species in the prepuce of some bulls interferes with the bacteriological diagnosis of *V. fetus* infection by cultivation from preputial washings, and that vaginal mucus culture from test heifers is the most exact method of diagnosis until a reliable selective medium can be found. Plastridge, Walker, Williams, Stula & Kiggins (342) give details of their method for the isolation of *V. fetus* from semen and preputial washings. A 4·0 mm. diameter loopful of semen plus a loopful of thiol medium is streaked over the surface of a fresh-blood agar plate, and also on a second plate two loopsful of a mixture of 3:1 semen to thiol. Preputial washings are dealt with in the same way both before and after centrifugation. The plates are incubated in a bell jar after 75% of its air has been removed and replaced by a mixture of CO_2 (10%) and nitrogen (85%). Morgan (343) has described methods for taking samples and has given details of media used for primary cultivation and for maintaining growth. He has also recorded details of the catalase test and the test for H_2S production. He concludes from his work that at present the only reliable method of diagnosis of infection in bulls is by test-mating on a vibrio-free heifer, culturing mucus from the anterior vagina and submitting the isolated vibrio to biochemical tests. Frank, Bryner & Caruthers (344) have isolated catalase-negative vibrios from bulls that have not been used for service, but they have not succeeded in isolating them from virgin heifers. They have not isolated catalase-positive vibrios from virgin heifers or from bulls that have not been used for

service. Robinson, van Rensburg, van Heerden & van Drimmelen (345) isolated *V. fetus* from bull semen by intraperitoneal injection of semen into pregnant guinea-pigs followed after a few days by culture of the foetal stomach contents. Some of the infected samples had been sent by rail (on ice) from 1000 miles away. Morgan (346) reports that attempts to use guinea-pigs for diagnosis of vibriosis in bulls failed. Götz (347) found that irrigation of the vagina with saline and subsequent culture did not detect all cows harbouring vibrios as shown by post-mortem examination.

Serological diagnosis. It is stated in the F.A.O. publication (318) that it is generally considered that the serum-agglutination test is of very limited value for diagnostic purposes, and that it is much inferior to the mucus-agglutination test. The serum-agglutination titres of infected animals are not regularly increased by the stage of the infection except possibly just after abortion, whereas the mucus titre is much more regularly increased whether or not abortion occurs. Mucus agglutinins appear at a varying period after first exposure to infection; estimates of their first appearance of 24–76 days and 40–60 days have been given. Agglutinins have been reported to appear as early as 14 days and as late as 105 days. It is necessary, therefore, to select with care the animals from which specimens are taken if all suspected cows and heifers cannot be sampled. Details are given of the method of obtaining specimens of mucus, the techique of the mucus-agglutination test and the production and standardization of antigen for the test. The mucus-agglutination test involves the use of 'Davis' agar as a permeable membrane for the extraction from the vaginal mucus of the antibodies with phenol-saline and the use of this extract in a tube agglutination test.

The value of the complement-fixation test in the diagnosis of *V. fetus* infection in cattle is discussed by Ertürk (348). Complement-fixation tests were done on sera from twenty cattle, most of which were infected. The antigen was prepared by treatment with methyl alcohol and acetone of cultures of *V. fetus* on thiol medium to which glutathiole (0·05 g./l.) and agar (20 g./l.) had been added. The results agreed with those of the agglutination test with blood and mucus. Trilenko (349) describes a slow complement-fixation test developed at the Leningrad Veterinary Research Institute, for the diagnosis of *V. fetus* infection in cattle. The serum to be tested is diluted with saline, inactivated by heating at 63° C. for 30 min. and then mixed with the antigen and the complement. This mixture is kept for 16–18 hr. at 0–4° C., and then allowed to regain room temperature before the haemolytic system, which has also been kept at 0–4° C. for 16–18 hr., is added. The mixture is heated to 37–38° C. and the results read in 20 min. A formolized suspension of *V. fetus* is used as antigen. When compared with the ordinary complement-fixation test, it was found that of 260 cows on infected farms, fourteen reacted to the ordinary test and forty-six to the slow test. Uniform results were obtained when the slow test was compared with the mucus-agglutination test, but in cows with chronic infection the tests did not always agree. The test can be used for semen.

D. *General*

In the F.A.O. publication on *Vibrio fetus* infection in cattle (318) there are sections referring to epidemiology and treatment, to management of established infection and to prevention and control of genital vibriosis. It is intended to refer here only to recent work relating to these aspects of the disease.

Incidence. It is reported by Weikl (350) that of 2100 samples of material from cases of abortion in cattle in Bavaria and Swabia since the end of 1954, 103 (5%) were found to contain *V. fetus*. Sporadic abortions in cattle caused by *V. fetus* have been reported in the province of Gdansk since 1955 according to Czarnowski (351). Dedié & Voigt (352) state that in 1954 the examination of foetuses, placentas and vaginal and preputial secretions reveal *V. fetus* infection in twenty-two districts in central Germany which relied on communal bulls, and in three artificial insemination centres in the Leipzig region. Jacotot & Vallée (353) report that a serological survey of about 1000 cattle in 16 Departments of France revealed *V. fetus* infection in 20%, and a further 25% gave suspect reactions. They (354) isolated eleven bovine strains from specimens from various parts of France. It is considered likely that vibrio infections will be demonstrated in other districts.

Pröger(355) reports on a statistical survey of 644 herds of cattle in Thuringia to assess the importance of *V. fetus* infection in bulls causing abortion and infertility in cows. He states that the organism was isolated from preputial washings and semen of young bulls not yet used for breeding and also from older bulls used with excellent results in natural service and artificial insemination, and that this suggests the presence in bulls of non-pathogenic strains of *V. fetus*. Most authorities would refer to these organisms as non-pathogenic vibrios and not by the specific name of *V. fetus*. Kawashima, Iwata & Suzuki(356) give a detailed review of abortion and infertility in cattle in Japan from 1936 to 1953. *V. fetus* was isolated from 143 of 561 aborted foetuses examined during this period. It was the cause of serious outbreaks of abortion and infertility in 1952/53 in which bulls played a major role in the spread of the infection. Using the mucus-agglutination test, Boyd(357) examined over 20,000 samples of vaginal mucus from more than 8000 cattle in 105 dairy herds in Sweden. Acute infection was diagnosed in nine herds, chronic active infection in thirty-six herds, chronic latent infection, where reactors were present, but no active cases, in eighteen herds. The remaining forty-two herds were not infected. This author points out that the mucus-agglutination test should be looked upon as a herd or group test and not as a test for infection in the individual animal. Mucus-agglutination tests on 2563 samples from cows and heifers in 113 herds in six northern counties of England were done by Hunter(358) and positive reactions were obtained in 13·7% of the samples. *V. fetus* infection was diagnosed in fifteen herds, and it was suspected in a further eight herds. The incidence of animals reacting positively to the test in herds in which vibriosis was not detectable clinically varied from 1 to 30%. It is considered that unless the reactors number at least 30–40% and there is also supporting clinical evidence of infection, the value of the mucus-agglutination test for the diagnosis of pathogenic vibriosis is doubtful.

The treatment of *V. fetus* infection in bulls, diagnosed as carriers by test-mating, is reported by Melrose, Morgan, Stewart & Thomson(359). They introduced into the prepuce, by means of a sterile catheter, 2 g. streptomycin and 1 mega-unit of penicillin made up to 100 ml. either in an oily base or as a cream. The prepuce was tied off with a gauze bandage which was left tied for an hour. It was massaged for 1 min. every 10 min. After treatment thirteen bulls were all negative on test mating, but catalase-negative vibrios were isolated from some of them. Tutt(360) suggests that asexual rest for 3 months is beneficial in the control of vibriosis. The resistance of *V. fetus* to different antibiotics and other therapeutic substances has been tested *in vitro* by Mundt(361). Concentrations of 1000 units of penicillin and 100 mg. of streptomycin per ml. of culture medium had no adverse effect on the growth of strain 29. Of ten other strains growth was inhibited in six by using 1000 units per ml. of penicillin. Streptomycin at 50 mg. per ml. inhibited growth of most strains but aureomycin did not. The same author(362) points out that because of the remarkable resistance of *V. fetus* to low temperatures, it is likely to survive the deep freezing of semen. Willett, Ohms, Frank, Bryner & Bartlett(363), however, report that storage of semen at 5° C. adversely affected *V. fetus*, even without the addition of antibiotics. They found that the addition of streptomycin to semen was as efficient as streptomycin with sulphanilamide or streptomycin with penicillin, in reducing the spread of *V. fetus* by infected bulls. Hughes(364) is of the opinion that the practice of adding antibiotics to the semen extender has aided in reducing the spread of vibriosis. On the other hand, Morgan, Melrose & Stewart(365) have shown that antibiotic in semen did not prevent heifers from becoming infected. DiLiello, Poelma & Faber(366) have made *in vitro* studies of the effect of antibiotics on *V. fetus* and on bovine spermatozoa. Many antibiotics were tried either alone or in combination, and it was found that dihydrostreptomycin at a level of 500 μg./ml. was the least toxic to spermatozoa and the most destructive to *V. fetus*. Wohanka(367) criticizes the belief that increased sterility amongst cattle in Germany has been caused by semen infected with *V. fetus* from artificial insemination centres. Nutritional factors and brucellosis are suggested as the cause. Referring to control of vibriosis, Blom(368,369) gives a preliminary report on a 5-year *V. fetus* control programme in breeding cattle in the Island of Møn, Denmark, using a modified tampon sampling technique for the mucus-agglutination test. This island, which has been free from tuberculosis and brucellosis

for some years, has an adult cattle population of 13,000, about 80–85% of which are of the Red Danish breed, the rest Jersey and Jersey crosses. About 98% of the Red Danish breed are artificially inseminated from *V. fetus*-free bulls on the island, the remainder by privately owned bulls. Jersey semen comes from bulls on the mainland, two of which were found to be infected in 1954. During the first 3 years of the programme, which envisages eradication, 24,909 mucus samples were tested. There were 188 (0·75%) positive reactions, representing 160 cattle in 95 herds, and 209 doubtful reactions. The incidence of reactors in Red Danish cattle in the 3 years was 0·4, 0·2 and 1·0%, but in Jersey cattle it was 7·5, 5·8 and 3·3%. There would seem to be good prospects of eradicating the infection if the Jersey bulls and the privately owned bulls were submitted to compulsory regular examination. Adler(370) discusses measures for the control of *V. fetus* infection in cattle in Denmark where 20% of the bulls kept in ninety artificial insemination centres were infected. The fact that 60% of the infected bulls had not been used for natural service is taken as evidence that direct transmission from bull to bull is possible. Control measures include the examination at regular intervals of all bulls kept at the centres and the disposal of those infected.

Papers on experimental investigations into vibriosis have been published during the period under review. Blobel, Simon & McNutt(371) submitted twenty-two heifers of an isolated brucellosis-free herd, to vaginal and intrauterine exposure to *V. fetus* culture. Only 71·4% of the animals exposed showed evidence of infection, as judged by the 'repeat breeding syndrome', recovery of organisms from uterine discharge and specific agglutinins in the vaginal mucus. No animals aborted. One pregnant heifer was fed a suspension of mixed strains of *V. fetus* on 2 consecutive days but did not become infected. The authors point out that the results may have been influenced by the use of laboratory strains of *V. fetus*, which had been kept for 2 years on laboratory medium. The work was of value in evaluating diagnostic procedures. The mucus-agglutination test proved a valuable aid to diagnosis provided samples were not collected at or near the time of oestrus. Agglutinins took 4–10 weeks to develop and persisted for 4–12 months. The diagnostic value of the serum-agglutination test appeared doubtful and agglutinins to *V. fetus* could not be demonstrated in the milk of a cow known to be infected. Conjunctival and intradermal injection of homologous antigens did not elicit any conclusive reactions. Simon & McNutt(372) found that the major histopathologic changes of experimentally produced vibriosis were salpingitis, endometritis and extensive generalized inflammatory oedema of the foetal membrane. Histological examination of the genitalia of a naturally infected cow made by Mundt(373) revealed desquamation of the epithelium, vascular engorgement and infiltration of the outer layers of the epithelium with leucocytes and lymphocytes. Plastridge, Easterbrooks, Williams, Kiggins & Walker(374) have studied the reproductive efficiency and serological findings in heifers experimentally infected with *V. fetus*. Infection, as shown by clinical evidence and the presence of agglutinins in the serum or mucus or both, followed cervical exposure in all of four heifers and vulval exposure in two of four heifers. Four to 12 weeks were required for the development of significant levels of agglutinins in the blood and mucus following cervical exposure. The infectivity of colonial variants of *V. fetus* strains for the chick embryo have been tested by White, Ristic & Sanders(375). With one exception smooth strains were more virulent than rough strains. A bovine 'saprophytic strain' showed a relatively high degree of infectivity compared with the other strains tested. The highest mortality was obtained with a smooth strain of human origin. There was no correlation between the number of viable cells inoculated and the embryo mortality. Pregnant and non-pregnant laboratory animals were inoculated with *V. fetus* by Iwata, Kawashima & Suzuki(376). Non-pregnant animals suffered no effect, but in pregnant animals abortion occurred as follows: 80% of those inoculated intravenously, 50% of those inoculated into the peritoneal cavity and 30% of those inoculated subcutaneously. The organism was recovered from the uterus, uterine discharges and the foetus. In male animals, inoculated by the intratesticular route, the organism survived for a long time in the testicles.

In conclusion it may be said that diagnosis of vibriosis depends almost entirely on the isolation of the organism as defined. Control of the disease, though appearing at first sight easy because

the spread is almost entirely venereal, is complicated by various factors such as the existence of carrier females and because the diagnosis of infection in bulls usually requires the use of test-mating.

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